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FILING DATE: *April 28, 2004*
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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15535 U.S. PTO 60/566212 042804

INVENTOR(S)		
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Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Methods of Generating Chimeric Adenoviruses and Uses For Such Chimeric Adenoviruses

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS (*check all that apply*)

☑ Specification *Number of Pages* 65
☑ Drawing(s) *Number of Sheets* 2
☐ Application Date Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify) 159 pp. Sequence Listing

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees.
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FILING FEE Amount ($): 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: ______

[Page 1 of 2]

Respectfully submitted,

SIGNATURE Cathy A. Kodroff

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Date April 28, 2004

REGISTRATION NO. 33,980
(if appropriate)
Docket Number: UPN-P3067-P2USA

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number	UPN-P3067-P2USA

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[Page 2 of 2]

Number 1 of 1

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☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 160.00

Complete if Known	
Application Number	
Filing Date	April 28, 2004
First Named Inventor	Roy et al
Examiner Name	
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Attorney Docket No.	UPN-P3067-P2USA

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

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FEE CALCULATION

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1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	160.00

SUBTOTAL (1) ($) 160.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X		=	
Independent Claims		- 3** =		X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify)

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SUBTOTAL (3) ($)

SUBMITTED BY

				(Complete *(if applicable)*)	
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METHODS OF GENERATING CHIMERIC ADENOVIRUSES AND USES FOR SUCH CHIMERIC ADENOVIRUSES

BACKGROUND OF THE INVENTION

The presence of humoral immunity (circulating antibodies) to adenovirus capsid proteins is a barrier to the use of adenovirus vectors for gene therapy. The prototype adenovirus vectors that have been developed for gene therapy are based on subgroup C adenoviruses such as that of serotype 5. The prevalence of neutralizing antibodies against subgroup C adenoviruses is generally high in human populations as a result of frequent exposure to these pathogens. This fact is likely to greatly limit the effectiveness of gene therapy vectors based on serotypes such as Ad5.

Analysis of the nature of the protective antibodies against adenoviruses has indicated that the most important target is the major capsid protein, hexon [Wolfhart (1988) *J. Virol* **62**, 2321; Gall *et al.* (1996) *J. Virol.* **70**, 2116]. Several efforts have been made to engineer the hexon so as to evade the anti-hexon antibodies by making chimeric adenoviruses harboring hexons from other serotypes [Roy *et al.* (1998) J. Virol. 72, 6875; US Patent No 5922315; Gall *et al.* (1998) *J. Virol.* **72**, 10260; Youil *et al.* (2002) *Hum. Gene Ther.* **13**, 311; Wu *et al.* (2002) *J. Virol.* **76**, 12775]. However, this has been largely unsuccessful when exchanges among distant serotypes are attempted.

Alternatively, investigators have proposed using adenovirus vectors that rarely cause human infections or using adenoviruses from non-human sources. However, the lack of a practical manner in which to produce large numbers of such vectors has proved to be a hindrance to developing such vectors.

SUMMARY OF THE INVENTION

The present invention provides a method of modifying adenoviruses having capsids, and particularly, including hexons, from serotypes which are not well adapted for growth in cells useful for adenoviral virion production. The method is useful for

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production of scalable amounts of adenoviruses. The modified, or chimeric, adenoviruses are useful for a variety of purposes which are described herein.

The invention further provides novel, isolated, adenovirus SA18 nucleic acid and amino acid sequences, vectors containing same, cell lines containing such SA18 sequences and/or vectors, and uses thereof.

Other aspects and advantages of the present invention will be readily apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 provides the map of the genome of the simian adenovirus generated by shotgun cloning as described in the examples below.

Fig. 2 provides the map of the recombinant Adhu5-SV25 chimeric virus. The portion of the genome replaced by DNA from Pan7 is indicated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides chimeric adenoviruses composed of the left terminal end and right terminal end of an adenovirus which can be cultured in the selected host cell, and the internal regions encoding, at a minimum, the capsid proteins of another adenovirus serotype. This invention is particularly advantageous for generating adenoviruses having serotypes which are difficult to culture in a desired cell type. The invention thus permits generation of chimeric adenoviruses of varying serotypes.

In the embodiments illustrated herein, chimeric adenoviruses have been constructed where most structural proteins, and not merely the hexon or fiber, are derived from an adenovirus of an unrelated serotype, thereby preserving the majority of the protein-protein interactions that are involved in capsid assembly. Most of the early genes such as those encoded by the adenovirus E1 and E4 regions that are responsible for transcription regulation and regulation of the host cell cycle, are retained from a different serotype that is known to result in high titer virus generation in the commonly used cell types, such as HEK 293 which supplies the Ad5 E1 proteins in *trans*.

In another embodiment, the invention provides novel nucleic acid and amino acid sequences from Ad SA18, which was originally isolated from vervet monkey

[ATCC VR-943]. The present invention further provides novel adenovirus vectors and packaging cell lines to produce those vectors for use in the *in vitro* production of recombinant proteins or fragments or other reagents. The invention further provides compositions for use in delivering a heterologous molecule for therapeutic or vaccine purposes. Such therapeutic or vaccine compositions contain the adenoviral vectors carrying an inserted heterologous molecule. In addition, novel sequences of the invention are useful in providing the essential helper functions required for production of recombinant adeno-associated viral (AAV) vectors. Thus, the invention provides helper constructs, methods and cell lines which use these sequences in such production methods.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the aligned sequences.

The term "substantial homology" or "substantial similarity," when referring to amino acids or fragments thereof, indicates that, when optimally aligned with appropriate amino acid insertions or deletions with another amino acid (or its complementary strand), there is amino acid sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or a protein thereof, or a fragment thereof which is at least 8 amino acids, or more desirably, at least 15 amino acids in length. Examples of suitable fragments are described herein.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome (e.g., about 36 kbp), the full-length of an open reading frame of a gene, protein, subunit, or enzyme [see, e.g., the tables providing the adenoviral coding regions], or a fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, e.g., of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Similarly, "percent sequence identity" may be readily determined for amino acid sequences,

over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length, and may be up to about 700 amino acids. Examples of suitable fragments are described herein.

Identity is readily determined using such algorithms and computer programs as are defined herein at default settings. Preferably, such identity is over the full length of the protein, enzyme, subunit, or over a fragment of at least about 8 amino acids in length. However, identity may be based upon shorter regions, where suited to the use to which the identical gene product is being put.

As described herein, alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs, such as "Clustal W", accessible through Web Servers on the internet. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Similarly programs are available for performing amino acid alignments. Generally, these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program that provides at least the level of identity or alignment as that provided by the referenced algorithms and programs.

As used throughout this specification and the claims, the term "comprise" and its variants including, "comprises", "comprising", among other variants, is inclusive of other components, elements, integers, steps and the like. The term "consists of" or "consisting of" are exclusive of other components, elements, integers, steps and the like.

Except where otherwise specified, the term "vector" includes any genetic element known in the art which will deliver a target molecule to a cell, including, naked DNA, a plasmid, phage, transposon, cosmids, episomes, viruses, etc.

By "minigene" is meant the combination of a selected heterologous gene and the other regulatory elements necessary to drive translation, transcription and/or expression of the gene product in a host cell.

As used herein, the term "transcomplement" refers to when a gene (gene product) of one adenovirus serotype supplies an adenovirus serotype lacking this gene (gene product) from another serotype with the missing function. For example, human adenovirus serotype 5 E1a and E1b functions are known to transcomplement E1-deleted chimpanzee adenovirus Pan 9. Similarly, the inventors have found that human Ad5 E1 transcomplements E1-deleted chimpanzee adenovirus serotypes Pan5, Pan6, Pan7, and simian adenovirus serotypes SV1, SV25 and SV39. Other examples of transcomplementing serotypes include human Ad5 and human Ad2, Ad3, Ad4, Ad5, Ad7, and Ad12.

The term "functionally deleted" or "functional deletion" means that a sufficient amount of the gene region is removed or otherwise damaged, e.g., by mutation or modification, so that the gene region is no longer capable of producing functional products of gene expression. If desired, the entire gene region may be removed. Other suitable sites for gene disruption or deletion are discussed elsewhere in the application.

The term "functional" refers to a product (e.g., a protein or peptide) which performs its native function, although not necessarily at the same level as the native product. The term "functional" may also refer to a gene which encodes and from which a desired product can be expressed.

I.Chimeric Adenoviral Vectors

The compositions of this invention include chimeric adenoviral vectors that deliver a heterologous molecule to cells. For delivery of such a heterologous molecule, the vector can be a plasmid or, preferably, a chimeric adenovirus. The chimeric adenoviruses of the invention include adenovirus DNA from at least two source serotypes, a "donating serotype" and a "parental adenovirus" as described in more detail herein, and a minigene.

Because the adenoviral genome contains open reading frames on both strands, in many instances reference is made herein to 5' and 3' ends of the various regions to avoid confusion between specific open reading frames and gene regions. Thus, when

reference is made herein to the "left" and "right" end of the adenoviral genome, this reference is to the ends of the approximately 36 kb adenoviral genome when depicted in schematic form as is conventional in the art [see, e.g., Horwitz, "Adenoviridae and Their Replication", in VIROLOGY, 2d ed., pp. 1679-1721 (1990)]. Thus, as used herein, the "left terminal end" of the adenoviral genome refers to portion of the adenoviral genome which, when the genome is depicted schematically in linear form, is located at the extreme left end of the schematic. Typically, the left end refers to be portion of the genome beginning at map unit 0 and extending to the right to include at least the 5' inverted terminal repeats (ITRs), and excludes the internal regions of the genome encoding the structural genes. As used herein, the "right terminal end" of the adenoviral genome refers to portion of the adenoviral genome which, when the genome is depicted schematically in linear form, is located at the extreme right end of the schematic. Typically, the right end of the adenoviral genome refers to be portion of the genome ending at map unit 36 and extending to the left to include at least the 3' ITRs, and excludes the internal regions of the genome encoding the structural genes.

A.Adenovirus Regulatory Sequences

1. Serotype

The selection of the adenovirus serotype donating its left terminal end and right terminal end can be readily made by one of skill in the art from among serotypes which can readily be cultured in the desired cell line. Among other factors which may be considered in selecting the serotype of the donating serotype is compatibility with the adenovirus serotype which will be supplying the internal regions at the location at which their sequences are hybridized.

Suitable adenoviruses for donating their left and right termini are available from the American Type Culture Collection, Manassas, Virginia, US (ATCC), a variety of academic and commercial sources, or the desired regions of the donating adenoviruses may be synthesized using known techniques with reference to sequences published in the literature or available from databases (e.g., GenBank, etc.). Examples of suitable donating adenoviruses include, without limitation, human adenovirus serotypes 2, 3, 4, 5, 7, and 12, and further including any of the presently identified human types [see, e.g., Horwitz, "Adenoviridae and Their Replication", in VIROLOGY, 2d ed., pp. 1679-1721 (1990)] which can be cultured in the desired cell. Similarly adenoviruses known to infect non-human primates (e.g., chimpanzees,

rhesus, macaque, and other simian species) or other non-human mammals and which grow in the desired cell can be employed in the vector constructs of this invention. Such serotypes include, without limitation, chimpanzee adenoviruses Pan 5 [VR-591], Pan6 [VR-592], Pan7 [VR-593], and C68 (Pan9), described in US Patent No. 6,083,716; and simian adenoviruses including, without limitation SV1 [VR-195]; SV25 [SV-201]; SV35; SV15; SV-34; SV-36; SV-37, and baboon adenovirus [VR-275], among others. In the following examples, the human 293 cells and adenovirus type 5 (Ad5), Pan9, and Ad40 are used for convenience. However, one of skill in the art will understand that other cell lines and/or comparable regions derived from other adenoviral strains may be readily selected and used in the present invention in the place of (or in combination with) these serotypes.

2. Sequences

The minimum sequences which must be supplied by the adenovirus donating its left terminal end and its right terminal end include the 5' cis-elements and the 3' cis-elements necessary for replication and packaging. Typically, the 5' cis-elements necessary for packaging and replication include the 5' inverted terminal repeat (ITR) sequences (which functions as origins of replication) and the native 5' packaging enhancer domains (that contain sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter). The right end of the adenoviral genome includes the 3' cis-elements (including the ITRs) necessary for packaging and encapsidation. Desirably, the adenovirus serotype donating its left and right termini and/or an adenovirus serotype which transcomplements the serotype of the donating adenovirus, further provides the functions of the necessary adenovirus early genes, including E1 (E1a and E1b), E2 (E2a and E2b), and E4 (including at least the ORF6 region). E3 is not essential and may be deleted as desired, e.g., for insertion of a transgene in this region or to provide space for a transgene inserted in another region (typically for packaging it is desirable for the total adenoviral genome to be under 36 kb).

In certain embodiments, the necessary adenovirus early genes are contained in the chimeric construct of the invention. In other embodiment, one or more of the necessary adenovirus early genes can be provided by the packaging host cell or in *trans*.

In general, the chimeric adenovirus of the invention contains regulatory sequences from the donating adenovirus serotype, or a transcomplementing serotype, to provide the chimeric adenovirus with compatible regulatory proteins. Optionally, one or more of the necessary adenoviral structural genes is provided by the adenovirus donating its left terminal and its right terminal end.

In certain embodiments, the chimeric adenovirus further contains one or more functional adenovirus genes, including, the Endoprotease open reading frame, DNA binding protein, 100 kDa scaffolding protein, 33 kDa protein, protein VIII, pTP, 52/55 kDa protein, protein VII, Mu and/or protein VI from the adenovirus serotype donating its left and right termini. Where all of these genes are derived from the adenovirus serotype donating the 5' and 3' ITRs, a "pseudotyped" chimeric is formed. Optionally, one or more of the genes can be hybrids formed from the fusion of the donating adenovirus serotype and the parental adenovirus serotype providing the capsid proteins (e.g., without limitation, polymerase, terminal protein, IIIa protein). Suitably, these genes express functional proteins which permit packaging of the adenovirus genes into the capsid. Alternatively, one or more of these proteins (whether hybrid or non-hybrid) can be functionally deleted in the chimeric adenovirus. Where desired, any necessary proteins functionally deleted in the chimeric adenovirus can be expressed in *trans* in the packaging cell.

B. Parental Adenovirus Structural Proteins

1. Serotypes

This invention is particularly well adapted for use in generating chimeric adenoviruses in which the capsid proteins are from a parental adenovirus which does not efficiently grow in a desirable host cell. The selection of the parental adenovirus serotype providing the internal regions can be readily made by one of skill in the art based on the information provided herein.

A variety of suitable adenoviruses can serve as a parental adenovirus supplying the regions encoding the structural (i.e., capsid proteins). Many of such adenoviruses can be obtained from the same sources as described above for the donating adenovirus serotypes. Examples of suitable parental adenovirus serotypes includes, without limitation, human adenovirus serotype 40, among others [see, e.g., Horwitz, "Adenoviridae and Their Replication", in VIROLOGY, 2d ed., pp. 1679-1721 (1990)], and adenoviruses known to infect non-human primates (e.g.,

chimpanzees, rhesus, macaque, and other simian species) or other non-human mammals, including, without limitation, chimpanzee adenovirus C1, described in US Patent No. 6,083,716; simian adenoviruses, and baboon adenoviruses, among others. In addition, the parental adenovirus supplying the internal regions may be from a non-naturally occurring adenovirus serotype, such as may be generated using a variety of techniques known to those of skill in the art.

In one embodiment illustrated herein, a chimeric virus that was constructed was that between the chimpanzee adenoviruses Pan-5 and C1 exhibited a higher titer in human 293 cells than the wild-type parental virus. However, the invention is not limited to the use of these chimpanzee adenoviruses, or to the combination of simian-simian, human-human, or simian-human chimeric adenoviruses. For example, it may be desirable to utilize bovine or canine adenoviruses, or other non-human mammalian adenoviruses which do not naturally infect and/or replicate in human cells.

In the following examples, the human adenovirus type 40 (Ad40) and the chimpanzee adenovirus C1, simian Pan 5 and Ad40, and Pan 5 and simian adenovirus SA18, are used. However, one of skill in the art will understand that other adenoviral serotypes may be readily selected and used in the present invention in the place of (or in combination with) these serotypes.

2. Sequences

The parental adenovirus provides to the chimeric construct of the invention its internal regions which includes structural proteins necessary for generating a capsid having the desired characteristics of the parental adenovirus. These desired characteristics include, but are not limited to, the ability to infect target cells and delivery a heterologous transgene, the ability to elude neutralizing antibodies directed to another adenovirus serotype (i.e., avoiding clearance due to cross-reactivity), and/or the ability to infect cells in the absence of an immune response to the chimeric adenovirus. The advantages of such characteristics may be most readily apparent in a regimen which involves repeat delivery of adenoviral vectors. The left and right termini of the parent adenovirus, including at least the 5' ITRs, the E1 region, the E4 region and the 3' ITRs are non-functional and, preferably, completely absent. Optionally, all adenovirus regulatory proteins from this parental adenovirus are non-functional and only the structural proteins (or selected structural proteins) are retained.

At a minimum, the parental adenovirus provides the adenoviral late region encoding the hexon protein. Suitably, the parental adenovirus further provides the late regions encoding the penton and the fiber. In certain embodiments, all of the functional adenoviral late regions, including L1 (encoding 52/55 Da, IIIa proteins), L2 (encoding penton, VII, V, Mu proteins), L3 (encoding VI, hexon, Endoprotease), L4 (encoding 100 kD, 33 kD, VIII proteins) and L5 (encoding fiber protein) are supplied by the parental adenovirus. Optionally, one or more of these late gene functions, with the exception of those encoding the hexon, penton and fiber proteins, can be functionally deleted. Any necessary structural proteins may be supplied in *trans*.

Thus, in certain embodiments, the chimeric adenovirus further contains one or more functional adenovirus genes, including, the Endoprotease open reading frame, DNA binding protein, 100 kDa scaffolding protein, 33 kDa protein, protein VIII, pTP, 52/55 kDa protein, protein VII, Mu and/or protein VI from the parental adenovirus donating its internal regions. Optionally, one or more of the genes can be hybrids formed from the fusion of the donating adenovirus serotype and the parental adenovirus serotype providing the capsid proteins, as described above.

C. The "Minigene"

Typically, an adenoviral vector of the invention is designed to contain a minigene which may be inserted into the site of a partially deleted, fully deleted (absent), or disrupted adenoviral gene. For example, the minigene may be located in the site of such a functional E1 deletion or functional E3 deletion, or another suitable site.

The methods employed for the selection of the transgene, the cloning and construction of the "minigene" and its insertion into the viral vector are within the skill in the art given the teachings provided herein.

1. The transgene

The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a host cell.

The composition of the transgene sequence will depend upon the use to which the adenoviral vector will be put. For example, the adenoviral vector may be used as a helper virus in production of recombinant adeno-associated viruses or in production of recombinant adenoviruses deleted of essential adenoviral gene functions which are supplied by the adenoviral vector, or for a variety of production uses. Alternatively, the adenoviral vector may be used for diagnostic purposes.

One type of transgene sequence includes a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc. These coding sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry. For example, where the marker sequence is the LacZ gene, the presence of the vector carrying the signal is detected by assays for beta-galactosidase activity. Where the transgene is GFP or luciferase, the vector carrying the signal may be measured visually by color or light production in a luminometer.

However, desirably, the transgene is a non-marker sequence encoding a product which is useful in biology and medicine, such as proteins, peptides, RNA, enzymes, or catalytic RNAs. Desirable RNA molecules include tRNA, dsRNA, ribosomal RNA, si RNAs, small hairpin RNAs, trans-splicing RNAs, catalytic RNAs, and antisense RNAs. One example of a useful RNA sequence is a sequence which extinguishes expression of a targeted nucleic acid sequence in the treated animal.

The transgene may be used for treatment, e.g., of genetic deficiencies, as a cancer therapeutic or vaccine, for induction of an immune response, and/or for prophylactic vaccine purposes. As used herein, induction of an immune response refers to the ability of a molecule (e.g., a gene product) to induce a T cell and/or a humoral immune response to the molecule. The invention further includes using multiple transgenes, e.g., to correct or ameliorate a condition caused by a multi-subunit protein. In certain situations, a different transgene may be used to encode each subunit of a protein, or to encode different peptides or proteins. This is desirable when the size of the DNA encoding the protein subunit is large, e.g., for an immunoglobulin, the platelet-derived growth factor, or a dystrophin protein. In order for the cell to produce the multi-subunit protein, a cell is infected with the recombinant virus containing each of the different subunits. Alternatively, different subunits of a protein may be encoded by the same transgene. In this case, a single transgene includes the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribozyme entry site (IRES). This is desirable when the size of the DNA encoding each of the subunits is small, e.g., the total size of the DNA encoding the subunits and the IRES is less than five kilobases. As an alternative to an IRES, the DNA may be separated by sequences encoding a 2A peptide, which self-cleaves in a post-translational event. See, e.g., M.L. Donnelly, *et al*, *J. Gen. Virol.*, 78(Pt 1):13-21 (Jan 1997); Furler, S., *et al*, *Gene Ther.*, 8(11):864-873 (June 2001); Klump H., *et al.*, *Gene Ther.*, 8(10):811-817 (May 2001). This 2A peptide is significantly smaller than an IRES, making it well suited for use when space is a limiting factor. However, the selected transgene may encode any biologically active product or other product, e.g., a product desirable for study.

Suitable transgenes may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention.

2. Vector and Transgene Regulatory Elements

In addition to the major elements identified above for the minigene, the adenoviral vector also includes conventional control elements which are operably linked to the transgene in a manner that permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked"

sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart *et al*, *Cell*, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1α promoter [Invitrogen].

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. For example, inducible promoters include the zinc-inducible sheep metallothionine (MT) promoter and the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter. Other inducible systems include the T7 polymerase promoter system [WO 98/10088]; the ecdysone insect promoter [No *et al*, *Proc. Natl. Acad. Sci. USA*, 93:3346-3351 (1996)], the tetracycline-repressible system [Gossen *et al*, *Proc. Natl. Acad. Sci. USA*, 89:5547-5551 (1992)], the tetracycline-inducible system [Gossen *et al*, *Science*, 268:1766-1769 (1995), see also Harvey *et al*, *Curr. Opin. Chem. Biol.*, 2:512-518 (1998)]. Other systems include the FK506 dimer, VP16 or p65 using castradiol, diphenol murislerone, the RU486-inducible system [Wang *et al*,

Nat. Biotech., 15:239-243 (1997) and Wang *et al*, *Gene Ther.*, 4:432-441 (1997)] and the rapamycin-inducible system [Magari *et al*, *J. Clin. Invest.*, 100:2865-2872 (1997)]. The effectiveness of some inducible promoters increases over time. In such cases one can enhance the effectiveness of such systems by inserting multiple repressors in tandem, e.g., TetR linked to a TetR by an IRES. Alternatively, one can wait at least 3 days before screening for the desired function. One can enhance expression of desired proteins by known means to enhance the effectiveness of this system. For example, using the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE).

In another embodiment, the native promoter for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

Another embodiment of the transgene includes a transgene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal β-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally occurring promoters (see Li *et al.*, Nat. Biotech., 17:241-245 (1999)). Examples of promoters that are tissue-specific are known for liver (albumin, Miyatake *et al.*, *J. Virol.*, 71:5124-32 (1997); hepatitis B virus core promoter, Sandig *et al.*, *Gene Ther.*, 3:1002-9 (1996); alpha-fetoprotein (AFP), Arbuthnot *et al.*, *Hum. Gene Ther.*, 7:1503-14 (1996)), bone osteocalcin (Stein *et al.*, *Mol. Biol. Rep.*, 24:185-96 (1997)); bone sialoprotein (Chen *et al.*, *J. Bone Miner. Res.*, 11:654-64 (1996)), lymphocytes (CD2, Hansal *et al.*, *J. Immunol.*, 161:1063-8 (1998); immunoglobulin heavy chain; T cell receptor chain), neuronal such as neuron-specific enolase (NSE) promoter (Andersen *et al.*, *Cell. Mol. Neurobiol.*, 13:503-15 (1993)), neurofilament light-chain gene (Piccioli *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:5611-5

(1991)), and the neuron-specific vgf gene (Piccioli *et al., Neuron,* 15:373-84 (1995)), among others.

Optionally, vectors carrying transgenes encoding therapeutically useful or immunogenic products may also include selectable markers or reporter genes may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. Such selectable reporters or marker genes (preferably located outside the viral genome to be packaged into a viral particle) can be used to signal the presence of the plasmids in bacterial cells, such as ampicillin resistance. Other components of the vector may include an origin of replication. Selection of these and other promoters and vector elements are conventional and many such sequences are available [see, e.g., Sambrook *et al*, and references cited therein].

These vectors are generated using the techniques and sequences provided herein, in conjunction with techniques known to those of skill in the art. Such techniques include conventional cloning techniques of cDNA such as those described in texts [Sambrook *et al*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY], use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence.

II. Production of the Recombinant Viral Particle

In one embodiment, the invention provides a method of generating recombinant chimeric adenoviral particles in which the capsid of the chimeric adenovirus is of a serotype incapable of efficient growth in the selected host cell. A vector suitable for production of recombinant chimeric adenoviral particles can be generated by direct cloning. Alternatively, such particles can be generated by homologous recombination between a first vector containing the left end of the chimeric adenoviral genome and a second vector containing the right end of the chimeric adenoviral genome. However, any suitable methodology known to those of skill in the art can be readily utilized to generate a vector suitable to generate a production vector, preferably which contains the entire chimeric adenoviral genome, including a minigene. This production vector is then introduced into a host cell in

which the adenoviral capsid protein is assembled and the chimeric adenoviral particle assembled as described.

The chimeric adenoviruses of the invention include those in which one or more adenoviral genes are absent, or otherwise rendered non-functional. If any of the missing gene functions are essential to the replication and infectivity of the adenoviral particle, these functions are supplied by a complementation (or transcomplementing) cell line or a helper vector expressing these functions during production of the chimeric adenoviral particle.

Examples of chimeric adenoviruses containing such missing adenoviral gene functions include those which are partially or completely deleted in the E1a and/or E1b gene. In such a case, the E1 gene functions can be supplied by the packaging host cell, permitting the chimeric construct to be deleted of E1 gene functions and, if desired, for a transgene to be inserted in this region. Optionally, the E1 gene can be of a serotype which transcomplements the serotype providing the other adenovirus sequences in order to further reduce the possibility of recombination and improve safety. In other embodiments, it is desirable to retain an intact E1a and/or E1b region in the recombinant adenoviruses. Such an intact E1 region may be located in its native location in the adenoviral genome or placed in the site of a deletion in the native adenoviral genome (e.g., in the E3 region).

In another example, all or a portion of the adenovirus delayed early gene E3 may be eliminated from the chimeric adenovirus. The function of adenovirus E3 is believed to be irrelevant to the function and production of the recombinant virus particle. Chimeric adenovirus vectors may also be constructed having a deletion of at least the ORF6 region of the E4 gene, and more desirably because of the redundancy in the function of this region, the entire E4 region. Still another vector of this invention contains a deletion in the delayed early gene E2a. Similarly, deletions in the intermediate genes IX and IVa_2 may be useful for some purposes. Optionally, deletions may also be made in selected portions of the late genes L1 through L5, as described above.

Other deletions may be made in the other structural or non-structural adenovirus genes. The above-discussed deletions may be used individually, i.e., an adenovirus sequence for use in the present invention may contain deletions in only a single region. Alternatively, deletions of entire genes or portions thereof effective to

destroy their biological activity may be used in any combination. For example, in one exemplary vector, the adenovirus sequence may have deletions of the E1 genes and the E4 gene, or of the E1, E2a and E3 genes, or of the E1 and E3 genes, or of E1, E2a and E4 genes, with or without deletion of E3, and so on. As discussed above, such deletions may be used in combination with other mutations, such as temperature-sensitive mutations, to achieve a desired result.

Examples of suitable transcomplementing serotypes are provided above. The use of transcomplementing serotypes can be particularly advantageous where there is diversity between the Ad sequences in the vector of the invention and the human AdE1 sequences found in currently available packaging cells. In such cases, the use of the current human E1-containing cells prevents the generation of replication-competent adenoviruses during the replication and production process. However, in certain circumstances, it will be desirable to utilize a cell line which expresses the E1 gene products can be utilized for production of an E1-deleted simian adenovirus. Such cell lines have been described. See, e.g., US Patent 6,083,716.

A. Packaging Host Cells

Suitably, the packaging host cell is selected from among cells in which the adenovirus serotype donating the left and right terminal ends of the chimeric genome are capable of efficient growth. The host cells are preferably of mammalian origin, and most preferably are of non-human primate or human origin.

Particularly desirable host cells are selected from among any mammalian species, including, without limitation, cells such as A549 [ATCC Accession No. CCL 185], 911 cells, WEHI, 3T3, 10T1/2, HEK 293 cells or PERC6 (both of which express functional adenoviral E1) [Fallaux, FJ *et al*, (1998), *Hum Gene Ther*, 9:1909-1917], Saos, C2C12, L cells, HT1080, HepG2, HeLa [ATCC Accession No. CCL 2], KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells, and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. These cell lines are all available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209. Other suitable cell lines may be obtained from other sources. The selection of the mammalian species providing the cells is not a limitation of this invention; nor is the type of mammalian cell, i.e., fibroblast, hepatocyte, tumor cell, etc.

As described above, a chimeric adenovirus of the invention can lack one or more functional adenoviral regulatory and/or structural genes which are supplied either by the host cell or in *trans* to effect packaging of the chimeric adenovirus into the viral capsid to generate the viral particle. Thus, the ability of a selected host cell to supply transcomplementing adenoviral sequences may be taken into consideration in selecting a desired host cell.

In one example, the cells are from a stable cell line which expresses adenovirus E1a and E1b functions from a cell line which transcomplements the adenovirus serotype which donates the left and right termini to the chimera of the invention, permitting the chimera to be E1-deleted. Alternatively, where the cell line does not transcomplement the adenovirus donating the termini, E1 functions may be provided by the chimera, or in trans.

If desired, one may utilize the sequences provided herein to generate a packaging cell or cell line that expresses, at a minimum, the adenovirus E1 gene from the adenovirus serotype donating the 5' ITR under the transcriptional control of a promoter for expression, or a transcomplementing serotype, in a selected parent cell line. Inducible or constitutive promoters may be employed for this purpose. Examples of such promoters are described in detail elsewhere in this specification. A parent cell is selected for the generation of a novel cell line expressing any desired adenovirus or adenovirus gene, including, e.g., a human Ad5, AdPan5, Pan6, Pan7, SV1, SV25 or SV39 gene. Without limitation, such a parent cell line may be HeLa [ATCC Accession No. CCL 2], A549 [ATCC Accession No. CCL 185], HEK 293, KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells, among others. Many of these cell lines are all available from the ATCC. Other suitable parent cell lines may be obtained from other sources.

Such E1-expressing cell lines are useful in the generation of chimeric adenovirus E1 deleted vectors. Additionally, or alternatively, the invention provides cell lines that express one or more simian adenoviral gene products, e.g., E1a, E1b, E2a, and/or E4 ORF6, can be constructed using essentially the same procedures for use in the generation of chimeric viral vectors. Such cell lines can be utilized to transcomplement adenovirus vectors deleted in the essential genes that encode those products, or to provide helper functions necessary for packaging of a helper-dependent virus (e.g., adeno-associated virus). The preparation of a host cell

according to this invention involves techniques such as assembly of selected DNA sequences. This assembly may be accomplished utilizing conventional techniques. Such techniques include cDNA and genomic cloning, which are well known and are described in Sambrook et al., cited above, use of overlapping oligonucleotide sequences of the adenovirus genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods which provide the desired nucleotide sequence.

In still another alternative, the essential adenoviral gene products are provided in *trans* by the adenoviral vector and/or helper virus. In such an instance, a suitable host cell can be selected from any biological organism, including prokaryotic (e.g., bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. Particularly desirable host cells are selected from among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, HEK 293 cells or PERC6 (both of which express functional adenoviral E1) [Fallaux, FJ et al, (1998), Hum Gene Ther, 9:1909-1917], Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. The selection of the mammalian species providing the cells is not a limitation of this invention; nor is the type of mammalian cell, i.e., fibroblast, hepatocyte, tumor cell, etc.

B. Helper Vectors

Thus, depending upon the adenovirus gene content of the adenoviral vectors and any adenoviral gene functions expressed from the host cell, a helper vector may be necessary to provide sufficient adenovirus gene sequences necessary to produce an infective recombinant viral particle containing the minigene. See, for example, the techniques described for preparation of a "minimal" human Ad vector in International Patent Application WO96/13597, published May 9, 1996, and incorporated herein by reference. Suitably, these helper vectors may be non-replicating genetic elements, a plasmid, or a virus.

Useful helper vectors contain selected adenovirus gene sequences not present in the adenovirus vector construct and/or not expressed by the packaging cell line in which the vector is transfected. In one embodiment, the helper virus is replication-defective and contains a variety of adenovirus genes in addition to the

sequences described above. Such a helper vector is desirably used in combination with an E1-expressing cell line.

Helper vectors may be formed into poly-cation conjugates as described in Wu *et al*, *J. Biol. Chem.*, 264:16985-16987 (1989); K. J. Fisher and J. M. Wilson, *Biochem. J.*, 299:49 (April 1, 1994). A helper vector may optionally contain a second reporter minigene. A number of such reporter genes are known to the art. The presence of a reporter gene on the helper virus which is different from the transgene on the adenovirus vector allows both the Ad vector and the helper vector to be independently monitored. This second reporter is used to enable separation between the resulting recombinant virus and the helper virus upon purification.

C. Assembly of Viral Particle and Transfection of a Cell Line

Generally, when delivering the vector comprising the minigene by transfection, the vector is delivered in an amount from about 5 µg to about 100 µg DNA, and preferably about 10 to about 50 µg DNA to about 1×10^4 cells to about 1×10^{13} cells, and preferably about 10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking into consideration such factors as the selected vector, the delivery method and the host cells selected.

Introduction into the host cell of the vector may be achieved by any means known in the art or as disclosed above, including transfection, and infection. One or more of the adenoviral genes may be stably integrated into the genome of the host cell, stably expressed as episomes, or expressed transiently. The gene products may all be expressed transiently, on an episome or stably integrated, or some of the gene products may be expressed stably while others are expressed transiently.

Furthermore, the promoters for each of the adenoviral genes may be selected independently from a constitutive promoter, an inducible promoter or a native adenoviral promoter. The promoters may be regulated by a specific physiological state of the organism or cell (i.e., by the differentiation state or in replicating or quiescent cells) or by exogenously added factors, for example.

Introduction of the molecules (as plasmids or viruses) into the host cell may also be accomplished using techniques known to the skilled artisan and as discussed throughout the specification. In preferred embodiment, standard transfection techniques are used, e.g., $CaPO_4$ transfection or electroporation.

Assembly of the selected DNA sequences of the adenovirus (as well as the transgene and other vector elements) into various intermediate plasmids, and the use of the plasmids and vectors to produce a recombinant viral particle are all achieved using conventional techniques. Such techniques include direct cloning as described [G. Gao *et al*, *Gene Ther.* 2003 Oct; **10**(22):1926-1930; US Patent Publication No. 2003-0092161-A, published May 15, 2003; International Patent Application No. PCT/US03/12405]. Other cloning techniques of cDNA such as those described in texts [Sambrook et al, cited above], use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence can be utilized. Standard transfection and co-transfection techniques are employed, *e.g.*, $CaPO_4$ precipitation techniques. Other conventional methods employed include homologous recombination of the viral genomes, plaquing of viruses in agar overlay, methods of measuring signal generation, and the like.

For example, following the construction and assembly of the desired minigene-containing viral vector, the vector is transfected in vitro in the presence of an optional helper vector into the packaging cell line. The functions expressed from the plasmid, packaging cell line and helper virus, if any, permits the adenovirus-transgene sequences in the vector to be replicated and packaged into virion capsids, resulting in the chimeric viral particles. The current method for producing such virus particles is transfection-based. However, the invention is not limited to such methods. The resulting chimeric adenoviruses are useful in transferring a selected transgene to a selected cell.

III. Use of the Chimeric Adenovirus Vectors

The chimeric adenovirus vectors of the invention are useful for gene transfer to a human or veterinary subject (including, non-human primates, non-simian primates, and other mammals) *in vitro*, *ex vivo*, and *in vivo*.

The recombinant adenovirus vectors described herein can be used as expression vectors for the production of the products encoded by the heterologous genes *in vitro*. For example, the recombinant adenoviruses containing a gene inserted into the location of an E1 deletion may be transfected into an E1-expressing cell line as described above. Alternatively, replication-competent adenoviruses may be used

in another selected cell line. The transfected cells are then cultured in the conventional manner, allowing the recombinant adenovirus to express the gene product from the promoter. The gene product may then be recovered from the culture medium by known conventional methods of protein isolation and recovery from culture.

A chimeric adenoviral vector of the invention provides an efficient gene transfer vehicle that can deliver a selected transgene to a selected host cell *in vivo* or *ex vivo* even where the organism has neutralizing antibodies to one or more AAV serotypes. In one embodiment, the rAAV and the cells are mixed *ex vivo*; the infected cells are cultured using conventional methodologies; and the transduced cells are re-infused into the patient. These compositions are particularly well suited to gene delivery for therapeutic purposes and for immunization, including inducing protective immunity.

More commonly, the chimeric adenoviral vectors of the invention will be utilized for delivery of therapeutic or immunogenic molecules, as described below. It will be readily understood for both applications that the recombinant adenoviral vectors of the invention are particularly well suited for use in regimens involving repeat delivery of recombinant adenoviral vectors. Such regimens typically involve delivery of a series of viral vectors in which the viral capsids are alternated. The viral capsids may be changed for each subsequent administration, or after a pre-selected number of administrations of a particular serotype capsid (e.g., one, two, three, four or more). Thus, a regimen may involve delivery of a rAd with a first capsid, delivery with a rAd with a second capsid, and delivery with a third capsid. A variety of other regimens which use the Ad capsids of the invention alone, in combination with one another, or in combination with other Ad serotypes will be apparent to those of skill in the art. Optionally, such a regimen may involve administration of rAd with capsids of non-human primate adenoviruses, human adenoviruses, or artificial (e.g., chimeric) serotypes such as are described herein. Each phase of the regimen may involve administration of a series of injections (or other delivery routes) with a single Ad serotype capsid followed by a series with another Ad serotype capsid. Alternatively, the recombinant Ad vectors of the invention may be utilized in regimens involving other non-adenoviral-mediated delivery systems, including other viral systems, non-viral delivery systems, protein, peptides, and other biologically active molecules.

The following sections will focus on exemplary molecules which may be delivered via the adenoviral vectors of the invention.

A. Ad-Mediated Delivery of Therapeutic Molecules

In one embodiment, the Ad vectors described herein are administered to humans according to published methods for gene therapy. A viral vector of the invention bearing the selected transgene may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous isotonic sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The adenoviral vectors are administered in sufficient amounts to transduce the target cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the retina and other intraocular delivery methods, direct delivery to the liver, inhalation, intranasal, intravenous, intramuscular, intratracheal, subcutaneous, intradermal, rectal, oral and other parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the transgene or the condition. The route of administration primarily will depend on the nature of the condition being treated.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective adult human or veterinary dosage of the viral vector is generally in the range of from about 100 μL to about 100 mL of a carrier containing concentrations of from about 1×10^{6} to about 1×10^{15} particles, about 1×10^{11} to 1×10^{13} particles, or about 1×10^{9} to 1×10^{12} particles. Dosages will range depending upon the size of the animal and the route of administration. For example, a suitable human or veterinary dosage (for about an 80 kg animal) for intramuscular injection is in the range of about 1×10^{9} to about 5×10^{12} particles per mL, for a single site. Optionally, multiple sites of administration may be delivered. In another example, a suitable human or veterinary dosage may be

in the range of about 1×10^{11} to about 1×10^{15} particles for an oral formulation. One of skill in the art may adjust these doses, depending the route of administration, and the therapeutic or vaccinal application for which the recombinant vector is employed. The levels of expression of the transgene, or for an immunogen, the level of circulating antibody, can be monitored to determine the frequency of dosage administration. Yet other methods for determining the timing of frequency of administration will be readily apparent to one of skill in the art.

An optional method step involves the co-administration to the patient, either concurrently with, or before or after administration of the viral vector, of a suitable amount of a short acting immune modulator. The selected immune modulator is defined herein as an agent capable of inhibiting the formation of neutralizing antibodies directed against the recombinant vector of this invention or capable of inhibiting cytolytic T lymphocyte (CTL) elimination of the vector. The immune modulator may interfere with the interactions between the T helper subsets (T_{H1} or T_{H2}) and B cells to inhibit neutralizing antibody formation. Alternatively, the immune modulator may inhibit the interaction between T_{H1} cells and CTLs to reduce the occurrence of CTL elimination of the vector. A variety of useful immune modulators and dosages for use of same are disclosed, for example, in Yang *et al.*, *J. Virol.*, 70(9) (Sept 1996); International Patent Application No. WO96/12406, published May 2, 1996; and International Patent Application No.PCT/US96/03035, all incorporated herein by reference. Typically, such immune modulators would be selected when the transgene is a therapeutic which requires repeat delivery.

1. Therapeutic Transgenes

Useful therapeutic products encoded by the transgene include hormones and growth and differentiation factors including, without limitation, insulin, glucagon, growth hormone (GH), parathyroid hormone (PTH), growth hormone releasing factor (GRF), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α (TGF α), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), any one of the

transforming growth factor superfamily, including TGF, activins, inhibins, or any of the bone morphogenic proteins (BMP) BMPs 1-15, any one of the heregluin/neuregulin/ARIA/neu differentiation factor (NDF) family of growth factors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3 and NT-4/5, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurturin, agrin, any one of the family of semaphorins/collapsins, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

Other useful transgene products include proteins that regulate the immune system including, without limitation, cytokines and lymphokines such as thrombopoietin (TPO), interleukins (IL) IL-1 through IL-25 (including, e.g., IL-2, IL-4, IL-12 and IL-18), monocyte chemoattractant protein, leukemia inhibitory factor, granulocyte-macrophage colony stimulating factor, Fas ligand, tumor necrosis factors and, interferons, and, stem cell factor, flk-2/flt3 ligand. Gene products produced by the immune system are also useful in the invention. These include, without limitation, immunoglobulins IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules, as well as engineered immunoglobulins and MHC molecules. Useful gene products also include complement regulatory proteins such as complement regulatory proteins, membrane cofactor protein (MCP), decay accelerating factor (DAF), CR1, CF2 and CD59.

Still other useful gene products include any one of the receptors for the hormones, growth factors, cytokines, lymphokines, regulatory proteins and immune system proteins. The invention encompasses receptors for cholesterol regulation, including the low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor, the very low density lipoprotein (VLDL) receptor, proteins useful in the regulation of lipids, including, e.g., apolipoprotein (apo) A and its isoforms (e.g., ApoAI), apoE and its isoforms including E2, E3 and E4), SRB1, ABC1, and the scavenger receptor. The invention also encompasses gene products such as members of the steroid hormone receptor superfamily including glucocorticoid receptors and estrogen receptors, Vitamin D receptors and other nuclear receptors. In addition, useful gene products include transcription factors such as jun, fos, max, mad, serum response factor (SRF), AP-1, AP2, myb, MyoD and

myogenin, ETS-box containing proteins, TFE3, E2F, ATF1, ATF2, ATF3, ATF4, ZF5, NFAT, CREB, HNF-4, C/EBP, SP1, CCAAT-box binding proteins, interferon regulation factor (IRF-1), Wilms tumor protein, ETS-binding protein, STAT, GATA-box binding proteins, e.g., GATA-3, and the forkhead family of winged helix proteins.

Other useful gene products include, carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, cystathione beta-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-coA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta-glucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, a cystic fibrosis transmembrane regulator (CFTR) sequence, and a dystrophin cDNA sequence. Other useful gene products include those useful for treatment of hemophilia A (e.g., Factor VIII and its variants, including the light chain and heavy chain of the heterodimer, optionally operably linked by a junction), and the B-domain deleted Factor VIII, see US 6,200,560 and 6,221,349], and useful for treatment of hemophilia B (e.g, Factor IX).

Still other useful gene products include non-naturally occurring polypeptides, such as chimeric or hybrid polypeptides having a non-naturally occurring amino acid sequence containing insertions, deletions or amino acid substitutions. For example, single-chain engineered immunoglobulins could be useful in certain immunocompromised patients. Other types of non-naturally occurring gene sequences include antisense molecules and catalytic nucleic acids, such as ribozymes, which could be used to reduce overexpression of a target.

Reduction and/or modulation of expression of a gene are particularly desirable for treatment of hyperproliferative conditions characterized by hyperproliferating cells, as are cancers and psoriasis. Target polypeptides include those polypeptides which are produced exclusively or at higher levels in hyperproliferative cells as compared to normal cells. Target antigens include polypeptides encoded by oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF. In addition to oncogene products as

target antigens, target polypeptides for anti-cancer treatments and protective regimens include variable regions of antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used as target antigens for autoimmune disease. Other tumor-associated polypeptides can be used as target polypeptides such as polypeptides which are found at higher levels in tumor cells including the polypeptide recognized by monoclonal antibody 17-1A and folate binding polypeptides.

Other suitable therapeutic polypeptides and proteins include those which may be useful for treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce self-directed antibodies. T-cell mediated autoimmune diseases include rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors (TCRs) that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases.

The chimeric adenoviral vectors of the invention are particularly well suited for therapeutic regimens in which multiple adenoviral-mediated deliveries of transgenes is desired, e.g., in regimens involving redelivery of the same transgene or in combination regimens involving delivery of other transgenes. Such regimens may involve administration of a chimeric adenoviral vector, followed by re-administration with a vector from the same serotype adenovirus. Particularly desirable regimens involve administration of a chimeric adenoviral vector of the invention, in which the serotype of the viral vector delivered in the first administration differs from the serotype of the viral vector utilized in one or more of the subsequent administrations. For example, a therapeutic regimen involves administration of a chimeric vector and repeat administration with one or more adenoviral vectors of the same or different serotypes. In another example, a therapeutic regimen involves administration of an adenoviral vector followed by repeat administration with a chimeric vector of the invention which differs from the serotype of the first delivered adenoviral vector, and optionally further administration with another vector which is the same or, preferably,

differs from the serotype of the vector in the prior administration steps. These regimens are not limited to delivery of adenoviral vectors constructed using the chimeric serotypes of the invention. Rather, these regimens can readily utilize chimeric or non-chimeric vectors of other adenoviral serotypes, which may be of artificial, human or non-human primate, or other mammalian sources, in combination with one or more of the chimeric vectors of the invention. Examples of such serotypes are discussed elsewhere in this document. Further, these therapeutic regimens may involve either simultaneous or sequential delivery of chimeric adenoviral vectors of the invention in combination with non-adenoviral vectors, non-viral vectors, and/or a variety of other therapeutically useful compounds or molecules. The present invention is not limited to these therapeutic regimens, a variety of which will be readily apparent to one of skill in the art.

B. Ad-Mediated Delivery of Immunogenic Transgenes

The adenoviruses of the invention may also be employed as immunogenic compositions. As used herein, an immunogenic composition is a composition to which a humoral (e.g., antibody) or cellular (e.g., a cytotoxic T cell) response is mounted to a transgene product delivered by the immunogenic composition following delivery to a mammal, and preferably a primate. The present invention provides an Ad that can contain in any of its adenovirus sequence deletions a gene encoding a desired immunogen. Chimeric adenoviruses based on simian or other non-human mammalian primate serotypes are likely to be better suited for use as a live recombinant virus vaccine in different animal species compared to an adenovirus of human origin, but is not limited to such a use. The recombinant adenoviruses can be used as prophylactic or therapeutic vaccines against any pathogen for which the antigen(s) crucial for induction of an immune response and able to limit the spread of the pathogen has been identified and for which the cDNA is available.

Such vaccinal (or other immunogenic) compositions are formulated in a suitable delivery vehicle, as described above. Generally, doses for the immunogenic compositions are in the range defined above for therapeutic compositions. The levels of immunity of the selected gene can be monitored to determine the need, if any, for boosters. Following an assessment of antibody titers in the serum, optional booster immunizations may be desired.

Optionally, a vaccinal composition of the invention may be formulated to contain other components, including, e.g. adjuvants, stabilizers, pH adjusters, preservatives and the like. Such components are well known to those of skill in the vaccine art. Examples of suitable adjuvants include, without limitation, liposomes, alum, monophosphoryl lipid A, and any biologically active factor, such as cytokine, an interleukin, a chemokine, a ligands, and optimally combinations thereof. Certain of these biologically active factors can be expressed in vivo, e.g., via a plasmid or viral vector. For example, such an adjuvant can be administered with a priming DNA vaccine encoding an antigen to enhance the antigen-specific immune response compared with the immune response generated upon priming with a DNA vaccine encoding the antigen only.

The adenoviruses are administered in "an immunogenic amount", that is, an amount of adenovirus that is effective in a route of administration to transfect the desired cells and provide sufficient levels of expression of the selected gene to induce an immune response. Where protective immunity is provided, the recombinant adenoviruses are considered to be vaccine compositions useful in preventing infection and/or recurrent disease.

Alternatively, or in addition, the vectors of the invention may contain a transgene encoding a peptide, polypeptide or protein which induces an immune response to a selected immunogen. The recombinant adenoviruses of this invention are expected to be highly efficacious at inducing cytolytic T cells and antibodies to the inserted heterologous antigenic protein expressed by the vector.

For example, immunogens may be selected from a variety of viral families. Example of desirable viral families against which an immune response would be desirable include, the picornavirus family, which includes the genera rhinoviruses, which are responsible for about 50% of cases of the common cold; the genera enteroviruses, which include polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as hepatitis A virus; and the genera apthoviruses, which are responsible for foot and mouth diseases, primarily in non-human animals. Within the picornavirus family of viruses, target antigens include the VP1, VP2, VP3, VP4, and VPG. Another viral family includes the calcivirus family, which encompasses the Norwalk group of viruses, which are an important causative agent of epidemic gastroenteritis. Still another viral family desirable for use in targeting antigens for

inducing immune responses in humans and non-human animals is the togavirus family, which includes the genera alphavirus, which include Sindbis viruses, RossRiver virus, and Venezuelan, Eastern & Western Equine encephalitis, and rubivirus, including Rubella virus. The flaviviridae family includes dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. Other target antigens may be generated from the Hepatitis C or the coronavirus family, which includes a number of non-human viruses such as infectious bronchitis virus (poultry), porcine transmissible gastroenteric virus (pig), porcine hemagglutinatin encephalomyelitis virus (pig), feline infectious peritonitis virus (cats), feline enteric coronavirus (cat), canine coronavirus (dog), and human respiratory coronaviruses, which may cause the common cold and/or non-A, B or C hepatitis. In addition, the human coronaviruses include the putative causative agent of sudden acute respiratory syndrome (SARS). Within the coronavirus family, target antigens include the E1 (also called M or matrix protein), E2 (also called S or Spike protein), E3 (also called HE or hemagglutin-elterose) glycoprotein (not present in all coronaviruses), or N (nucleocapsid). Still other antigens may be targeted against the rhabdovirus family, which includes the genera vesiculovirus (e.g., Vesicular Stomatitis Virus), and the general lyssavirus (e.g., rabies). Within the rhabdovirus family, suitable antigens may be derived from the G protein or the N protein. The family filoviridae, which includes hemorrhagic fever viruses such as Marburg and Ebola virus, may be a suitable source of antigens. The paramyxovirus family includes parainfluenza Virus Type 1, parainfluenza Virus Type 3, bovine parainfluenza Virus Type 3, rubulavirus (mumps virus), parainfluenza Virus Type 2, parainfluenza virus Type 4, Newcastle disease virus (chickens), rinderpest, morbillivirus, which includes measles and canine distemper, and pneumovirus, which includes respiratory syncytial virus. The influenza virus is classified within the family orthomyxovirus and is a suitable source of antigen (e.g., the HA protein, the N1 protein). The bunyavirus family includes the genera bunyavirus (California encephalitis, La Crosse), phlebovirus (Rift Valley Fever), hantavirus (puremala is a hemahagin fever virus), nairovirus (Nairobi sheep disease) and various unassigned bungaviruses. The arenavirus family provides a source of antigens against LCM and Lassa fever virus. The reovirus family includes the genera reovirus, rotavirus (which

causes acute gastroenteritis in children), orbiviruses, and cultivirus (Colorado Tick fever), Lebombo (humans), equine encephalosis, blue tongue.

The retrovirus family includes the sub-family oncorivirinal which encompasses such human and veterinary diseases as feline leukemia virus, HTLVI and HTLVII, lentivirinal (which includes human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus, and spumavirinal). Among the lentiviruses, many suitable antigens have been described and can readily be selected. Examples of suitable HIV and SIV antigens include, without limitation the gag, pol, Vif, Vpx, VPR, Env, Tat, Nef, and Rev proteins, as well as various fragments thereof. For example, suitable fragments of the Env protein may include any of its subunits such as the gp120, gp160, gp41, or smaller fragments thereof, e.g., of at least about 8 amino acids in length. Similarly, fragments of the tat protein may be selected. [See, US Patent 5,891,994 and US Patent 6,193,981.] See, also, the HIV and SIV proteins described in D.H. Barouch *et al*, *J. Virol.*, 75(5):2462-2467 (March 2001), and R.R. Amara, *et al*, *Science*, 292:69-74 (6 April 2001). In another example, the HIV and/or SIV immunogenic proteins or peptides may be used to form fusion proteins or other immunogenic molecules. See, e.g., the HIV-1 Tat and/or Nef fusion proteins and immunization regimens described in WO 01/54719, published August 2, 2001, and WO 99/16884, published April 8, 1999. The invention is not limited to the HIV and/or SIV immunogenic proteins or peptides described herein. In addition, a variety of modifications to these proteins have been described or could readily be made by one of skill in the art. See, *e.g.*, the modified gag protein that is described in US Patent 5,972,596. Further, any desired HIV and/or SIV immunogens may be delivered alone or in combination. Such combinations may include expression from a single vector or from multiple vectors. Optionally, another combination may involve delivery of one or more expressed immunogens with delivery of one or more of the immunogens in protein form. Such combinations are discussed in more detail below.

The papovavirus family includes the sub-family polyomaviruses (BKU and JCU viruses) and the sub-family papillomavirus (associated with cancers or malignant progression of papilloma). The adenovirus family includes viruses (EX, AD7, ARD, O.B.) which cause respiratory disease and/or enteritis. The parvovirus includes family feline parvovirus (feline enteritis), feline panleucopeniavirus, canine

parvovirus, and porcine parvovirus. The herpesvirus family includes the sub-family alphaherpesvirinae, which encompasses the genera simplexvirus (HSVI, HSVII), varicellovirus (pseudorabies, varicella zoster) and the sub-family betaherpesvirinae, which includes the genera cytomegalovirus (HCMV, muromegalovirus) and the sub-family gammaherpesvirinae, which includes the genera lymphocryptovirus, EBV (Burkitts lymphoma), infectious rhinotracheitis, Marek's disease virus, and rhadinovirus. The poxvirus family includes the sub-family chordopoxvirinae, which encompasses the genera orthopoxvirus (Variola (Smallpox) and Vaccinia (Cowpox)), parapoxvirus, avipoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, and the sub-family entomopoxvirinae. The hepadnavirus family includes the Hepatitis B virus. One unclassified virus which may be suitable source of antigens is the Hepatitis delta virus. Still other viral sources may include avian infectious bursal disease virus and porcine respiratory and reproductive syndrome virus. The alphavirus family includes equine arteritis virus and various Encephalitis viruses.

The viruses of the present invention may also carry immunogens which are useful to immunize a human or non-human animal against other pathogens including bacteria, fungi, parasitic microorganisms or multicellular parasites which infect human and non-human vertebrates, or from a cancer cell or tumor cell. Examples of bacterial pathogens include pathogenic gram-positive cocci include pneumococci; staphylococci; and streptococci. Pathogenic gram-negative cocci include meningococcus; gonococcus. Pathogenic enteric gram-negative bacilli include enterobacteriaceae; pseudomonas, acinetobacteria and eikenella; melioidosis; salmonella; shigella; haemophilus; moraxella; *H. ducreyi* (which causes chancroid); brucella; *Franisella tularensis* (which causes tularemia); yersinia (pasteurella); streptobacillus moniliformis and spirillum; Gram-positive bacilli include listeria monocytogenes; erysipelothrix rhusiopathiae; *Corynebacterium diphtheria* (diphtheria); cholera; *B. anthracis* (anthrax); donovanosis (granuloma inguinale); and bartonellosis. Diseases caused by pathogenic anaerobic bacteria include tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include syphilis; treponematoses: yaws, pinta and endemic syphilis; and leptospirosis. Other infections caused by higher pathogen bacteria and pathogenic fungi include actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis;

sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma and chromomycosis; and dermatophytosis. Rickettsial infections include Typhus fever, Rocky Mountain spotted fever, Q fever, and Rickettsialpox. Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections. Pathogenic eukaryotes encompass pathogenic protozoans and helminths and infections produced thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; *Pneumocystis carinii*; Trichans; *Toxoplasma gondii*; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections.

Many of these organisms and/or toxins produced thereby have been identified by the Centers for Disease Control [(CDC), Department of Heath and Human Services, USA], as agents which have potential for use in biological attacks. For example, some of these biological agents, include, *Bacillus anthracis* (anthrax), *Clostridium botulinum* and its toxin (botulism), *Yersinia pestis* (plague), variola major (smallpox), *Francisella tularensis* (tularemia), and viral hemorrhagic fevers [filoviruses (e.g., Ebola, Marburg], and arenaviruses [e.g., Lassa, Machupo]), all of which are currently classified as Category A agents; *Coxiella burnetti* (Q fever); Brucella species (brucellosis), *Burkholderia mallei* (glanders), *Burkholderia pseudomallei* (meloidosis), *Ricinus communis* and its toxin (ricin toxin), *Clostridium perfringens* and its toxin (epsilon toxin), Staphylococcus species and their toxins (enterotoxin B), *Chlamydia psittaci* (psittacosis), water safety threats (e.g., *Vibrio cholerae*, *Crytosporidium parvum*), Typhus fever (*Richettsia powazekii*), and viral encephalitis (alphaviruses, e.g., Venezuelan equine encephalitis; eastern equine encephalitis; western equine encephalitis); all of which are currently classified as Category B agents; and Nipan virus and hantaviruses, which are currently classified as Category C agents. In addition, other organisms, which are so classified or differently classified, may be identified and/or used for such a purpose in the future. It will be readily understood that the viral vectors and other constructs described herein are useful to deliver antigens from these organisms, viruses, their toxins or other by-products, which will prevent and/or treat infection or other adverse reactions with these biological agents.

Administration of the vectors of the invention to deliver immunogens against the variable region of the T cells elicit an immune response including CTLs to eliminate those T cells. In RA, several specific variable regions of TCRs which are involved in the disease have been characterized. These TCRs include V-3, V-14, V-17 and Vα-17. Thus, delivery of a nucleic acid sequence that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in RA. In MS, several specific variable regions of TCRs which are involved in the disease have been characterized. These TCRs include V-7 and Vα-10. Thus, delivery of a nucleic acid sequence that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in MS. In scleroderma, several specific variable regions of TCRs which are involved in the disease have been characterized. These TCRs include V-6, V-8, V-14 and Vα-16, Vα-3C, Vα-7, Vα-14, Vα-15, Vα-16, Vα-28 and Vα-12. Thus, delivery of a chimeric adenovirus that encodes at least one of these polypeptides will elicit an immune response that will target T cells involved in scleroderma.

C. Ad-Mediated Delivery Methods

The therapeutic levels, or levels of immunity, of the selected gene can be monitored to determine the need, if any, for boosters. Following an assessment of CD8+ T cell response, or optionally, antibody titers, in the serum, optional booster immunizations may be desired. Optionally, the adenoviral vectors of the invention may be delivered in a single administration or in various combination regimens, *e.g.*, in combination with a regimen or course of treatment involving other active ingredients or in a prime-boost regimen. A variety of such regimens have been described in the art and may be readily selected.

For example, prime-boost regimens may involve the administration of a DNA (e.g., plasmid) based vector to prime the immune system to a second or further, booster, administration with a traditional antigen, such as a protein or a recombinant virus carrying the sequences encoding such an antigen. See, *e.g.*, WO 00/11140, published March 2, 2000, incorporated by reference. Alternatively, an immunization regimen may involve the administration of a chimeric adenoviral vector of the invention to boost the immune response to a vector (either viral or DNA-based) carrying an antigen, or a protein. In still another alternative, an immunization

regimen involves administration of a protein followed by booster with a vector encoding the antigen.

In one embodiment, the invention provides a method of priming and boosting an immune response to a selected antigen by delivering a plasmid DNA vector carrying said antigen, followed by boosting with an adenoviral vector of the invention. In one embodiment, the prime-boost regimen involves the expression of multiproteins from the prime and/or the boost vehicle. See, e.g., R.R. Amara, *Science*, 292:69-74 (6 April 2001) which describes a multiprotein regimen for expression of protein subunits useful for generating an immune response against HIV and SIV. For example, a DNA prime may deliver the Gag, Pol, Vif, VPX and Vpr and Env, Tat, and Rev from a single transcript. Alternatively, the SIV Gag, Pol and HIV-1 Env is delivered in a recombinant adenovirus construct of the invention. Still other regimens are described in WO 99/16884 and WO 01/54719.

However, the prime-boost regimens are not limited to immunization for HIV or to delivery of these antigens. For example, priming may involve delivering with a first vector of the invention followed by boosting with a second vector, or with a composition containing the antigen itself in protein form. In one example, the prime-boost regimen can provide a protective immune response to the virus, bacteria or other organism from which the antigen is derived. In another desired embodiment, the prime-boost regimen provides a therapeutic effect that can be measured using convention assays for detection of the presence of the condition for which therapy is being administered.

The priming composition may be administered at various sites in the body in a dose dependent manner, which depends on the antigen to which the desired immune response is being targeted. The invention is not limited to the amount or situs of injection(s) or to the pharmaceutical carrier. Rather, the regimen may involve a priming and/or boosting step, each of which may include a single dose or dosage that is administered hourly, daily, weekly or monthly, or yearly. As an example, the mammals may receive one or two doses containing between about 10 µg to about 50 µg of plasmid in carrier. A desirable amount of a DNA composition ranges between about 1 µg to about 10,000 µg of the DNA vector. Dosages may vary from about 1 µg to 1000 µg DNA per kg of subject body weight. The amount or site of delivery is desirably selected based upon the identity and condition of the mammal.

The dosage unit of the vector suitable for delivery of the antigen to the mammal is described herein. The vector is prepared for administration by being suspended or dissolved in a pharmaceutically or physiologically acceptable carrier such as isotonic saline; isotonic salts solution or other formulations that will be apparent to those skilled in such administration. The appropriate carrier will be evident to those skilled in the art and will depend in large part upon the route of administration. The compositions of the invention may be administered to a mammal according to the routes described above, in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and liposomes. Optionally, the priming step of this invention also includes administering with the priming composition, a suitable amount of an adjuvant, such as are defined herein.

Preferably, a boosting composition is administered about 2 to about 27 weeks after administering the priming composition to the mammalian subject. The administration of the boosting composition is accomplished using an effective amount of a boosting composition containing or capable of delivering the same antigen as administered by the priming DNA vaccine. The boosting composition may be composed of a recombinant viral vector derived from the same viral source (e.g., adenoviral sequences of the invention) or from another source. Alternatively, the "boosting composition" can be a composition containing the same antigen as encoded in the priming DNA vaccine, but in the form of a protein or peptide, which composition induces an immune response in the host. In another embodiment, the boosting composition contains a DNA sequence encoding the antigen under the control of a regulatory sequence directing its expression in a mammalian cell, e.g., vectors such as well-known bacterial or viral vectors. The primary requirements of the boosting composition are that the antigen of the composition is the same antigen, or a cross-reactive antigen, as that encoded by the priming composition.

In another embodiment, the adenoviral vectors of the invention are also well suited for use in a variety of other immunization and therapeutic regimens. Such regimens may involve delivery of adenoviral vectors of the invention simultaneously or sequentially with Ad vectors of different serotype capsids, regimens in which adenoviral vectors of the invention are delivered simultaneously or sequentially with non-Ad vectors, regimens in which the adenoviral vectors of the invention are delivered simultaneously or sequentially with proteins, peptides, and/or

other biologically useful therapeutic or immunogenic compounds. Such uses will be readily apparent to one of skill in the art.

IV. Simian Adenovirus 18 Sequences

The invention provides nucleic acid sequences and amino acid sequences of Ad SA18, which are isolated from the other viral material with which they are associated in nature. These sequences are useful in preparing heterologous molecules containing the nucleic acid sequences and amino acid sequences, and regions or fragments thereof as are described herein, viral vectors which are useful for a variety of purposes, including the constructs and compositions, and such methods as are described herein for the chimeric adenoviruses, including, e.g., in host cells for production of viruses requiring adenoviral helper functions, as delivery vehicles for heterologous molecules such as those described herein. These sequences are also useful in generating the chimeric adenoviruses of the invention.

A. Nucleic Acid Sequences

The SA18 nucleic acid sequences of the invention include nucleotides SEQ ID NO: 12, nt 1 to 31967. See, Sequence Listing, which is incorporated by reference herein. The nucleic acid sequences of the invention further encompass the strand which is complementary to the sequences of SEQ ID NO: 12, as well as the RNA and cDNA sequences corresponding to the sequences of these sequences figures and their complementary strands. Further included in this invention are nucleic acid sequences which are greater than 95 to 98%, and more preferably about 99 to 99.9% homologous or identical to the Sequence Listing. Also included in the nucleic acid sequences of the invention are natural variants and engineered modifications of the sequences provided in SEQ ID NO: 12 and their complementary strands. Such modifications include, for example, labels that are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with a degenerate nucleotide.

The invention further encompasses fragments of the sequences of SA18, their complementary strand, cDNA and RNA complementary thereto. Suitable fragments are at least 15 nucleotides in length, and encompass functional fragments, i.e., fragments which are of biological interest. For example, a functional fragment can express a desired adenoviral product or may be useful in production of

recombinant viral vectors. Such fragments include the gene sequences and fragments listed in the tables below.

The following tables provide the transcript regions and open reading frames in the simian adenovirus sequences of the invention. For certain genes, the transcripts and open reading frames (ORFs) are located on the strand complementary to that presented in SEQ ID NO: 12. See, e.g., E2b, E4 and E2a. The calculated molecular weights of the encoded proteins are also shown.

Adenovirus Gene Region	Protein	Ad SA18, SEQ ID NO:12		
		start	End	M.W.
ITR		1	180	
E1a	13S	916	1765	27264
	12S	916	1765	24081
E1b	Small T	1874	2380	19423
	LargeT	2179	3609	52741
	IX	3678	4079	13701
E2b	IVa2	5478	4126	51295
	Polymerase	13745	5229	128392
	PTP	13745	8597	75358
	Agnoprotein	8007	8705	23610
L1	52/55 kD	10788	11945	43416
	IIIa	11966	13699	63999
L2	Penton	13796	15322	57166
	VII	15328	15873	20352
	V	15920	17050	42020
L3	VI	17348	18154	29222
	Hexon	18257	21010	102912
	Endoprotease	21029	21640	23015

Adenovirus Gene Region	Protein	Ad SA18, SEQ ID NO:12		
2a	DBP	23147	21711	53626
L4	100kD	23175	25541	87538
	22 kD homolog	25204	25797	22206
	33 kD homolog	25204	26025	24263
	VIII	26107	26817	25490
E3	Orf #1	26817	27125	11814
L5	Fiber	27192	29015	65455
E4	Orf 6/7	30169	29067	13768
	Orf 6	30169	29303	33832
	Orf 4	30464	30099	14154
	Orf 3	30816	30466	13493
	Orf 2	31205	30813	14698
	Orf 1	31608	31231	14054
ITR		31788	31967	

The SA18 adenoviral nucleic acid sequences are useful as therapeutic and immunogenic agents and in construction of a variety of vector systems and host cells. Such vectors are useful for any of the purposes described above for the chimeric adenovirus. Additionally, these SA18 sequences and products may be used alone or in combination with other adenoviral sequences or fragments, or in combination with elements from other adenoviral or non-adenoviral sequences. The adenoviral sequences of the invention are also useful as antisense delivery vectors, gene therapy vectors, or vaccine vectors, and in methods of using same. Thus, the invention further provides nucleic acid molecules, gene delivery vectors, and host cells which contain the Ad sequences of the invention.

For example, the invention encompasses a nucleic acid molecule containing simian Ad ITR sequences of the invention. In another example, the invention provides a nucleic acid molecule containing simian Ad sequences of the

invention encoding a desired Ad gene product. Still other nucleic acid molecule constructed using the sequences of the invention will be readily apparent to one of skill in the art, in view of the information provided herein.

In one embodiment, the simian Ad gene regions identified herein may be used in a variety of vectors for delivery of a heterologous molecule to a cell. Examples of such molecules and methods of delivery are provided in Section III herein. For example, vectors are generated for expression of an adenoviral capsid protein (or fragment thereof) for purposes of generating a viral vector in a packaging host cell. Such vectors may be designed for expression in trans. Alternatively, such vectors are designed to provide cells which stably contain sequences which express desired adenoviral functions, e.g., one or more of E1a, E1b, the terminal repeat sequences, E2a, E2b, E4, E4ORF6 region.

In addition, the adenoviral gene sequences and fragments thereof are useful for providing the helper functions necessary for production of helper-dependent viruses (e.g., adenoviral vectors deleted of essential functions or adeno-associated viruses (AAV)). For such production methods, the simian adenoviral sequences of the invention are utilized in such a method in a manner similar to those described for the human Ad. However, due to the differences in sequences between the simian adenoviral sequences of the invention and those of human Ad, the use of the sequences of the invention essentially eliminate the possibility of homologous recombination with helper functions in a host cell carrying human Ad E1 functions, e.g., 293 cells, which may produce infectious adenoviral contaminants during rAAV production.

Methods of producing rAAV using adenoviral helper functions have been described at length in the literature with human adenoviral serotypes: See, e.g., US Patent 6,258,595 and the references cited therein. See, also, US Patent 5,871,982; WO 99/14354; WO 99/15685; WO 99/47691. These methods may also be used in production of non-human serotype AAV, including non-human primate AAV serotypes. The simian adenoviral gene sequences of the invention which provide the necessary helper functions (e.g., E1a, E1b, E2a and/or E4 ORF6) can be particularly useful in providing the necessary adenoviral function while minimizing or eliminating the possibility of recombination with any other adenoviruses present in the rAAV-packaging cell which are typically of human origin. Thus, selected genes or open

reading frames of the adenoviral sequences of the invention may be utilized in these rAAV production methods.

Alternatively, recombinant adenoviral simian vectors of the invention may be utilized in these methods. Such recombinant adenoviral simian vectors may include, e.g., a hybrid simian Ad/AAV in which simian Ad sequences flank a rAAV expression cassette composed of, e.g., AAV 3' and/or 5' ITRs and a transgene under the control of regulatory sequences which control its expression. One of skill in the art will recognize that still other simian adenoviral vectors and/or gene sequences of the invention will be useful for production of rAAV and other viruses dependent upon adenoviral helper.

In still another embodiment, nucleic acid molecules are designed for delivery and expression of selected adenoviral gene products in a host cell to achieve a desired physiologic effect. For example, a nucleic acid molecule containing sequences encoding an adenovirus E1a protein of the invention may be delivered to a subject for use as a cancer therapeutic. Optionally, such a molecule is formulated in a lipid-based carrier and preferentially targets cancer cells. Such a formulation may be combined with other cancer therapeutics (e.g., cisplatin, taxol, or the like). Still other uses for the adenoviral sequences provided herein will be readily apparent to one of skill in the art.

In addition, one of skill in the art will readily understand that the Ad sequences of the invention can be readily adapted for use for a variety of viral and non-viral vector systems for in vitro, ex vivo or in vivo delivery of therapeutic and immunogenic molecules, including any of those identified as being deliverable via the chimeric adenoviruses of the invention. For example, the simian Ad genome of the invention can be utilized in a variety of rAd and non-rAd vector systems. Such vectors systems may include, e.g., plasmids, lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adeno-associated viral systems, among others. Selection of these vector systems is not a limitation of the present invention.

The invention further provides molecules useful for production of the simian and simian-derived proteins of the invention. Such molecules which carry polynucleotides including the simian Ad DNA sequences of the invention can be in the form of a vector.

B. Simian Adenoviral Proteins of the Invention

The invention further provides gene products of the above adenoviruses, such as proteins, enzymes, and fragments thereof, which are encoded by the adenoviral nucleic acids of the invention. The invention further encompasses SA18 proteins, enzymes, and fragments thereof, having the amino acid sequences encoded by these nucleic acid sequences which are generated by other methods. Such proteins include those encoded by the open reading frames identified in the tables above, and fragments thereof.

Thus, in one aspect, the invention provides unique simian adenoviral proteins which are substantially pure, i.e., are free of other viral and proteinaceous proteins. Preferably, these proteins are at least 10% homogeneous, more preferably 60% homogeneous, and most preferably 95% homogeneous.

In one embodiment, the invention provides unique simian-derived capsid proteins. As used herein, a simian-derived capsid protein includes any adenoviral capsid protein that contains a SA18 capsid protein or a fragment thereof, as defined above, including, without limitation, chimeric capsid proteins, fusion proteins, artificial capsid proteins, synthetic capsid proteins, and recombinantly capsid proteins, without limitation to means of generating these proteins.

Suitably, these simian-derived capsid proteins contain one or more SA18 regions or fragments thereof (e.g., a hexon, penton, fiber or fragment thereof) in combination with capsid regions or fragments thereof of different adenoviral serotypes, or modified simian capsid proteins or fragments, as described herein. A "modification of a capsid protein associated with altered tropism" as used herein includes an altered capsid protein, i.e, a penton, hexon or fiber protein region, or fragment thereof, such as the knob domain of the fiber region, or a polynucleotide encoding same, such that specificity is altered. The simian-derived capsid may be constructed with one or more of the simian Ad of the invention or another Ad serotypes which may be of human or non-human origin. Such Ad may be obtained from a variety of sources including the ATCC, commercial and academic sources, or the sequences of the Ad may be obtained from GenBank or other suitable sources.

The amino acid sequences of the simian adenoviruses penton proteins of the invention are provided herein. The AdSA18 penton protein is provided in SEQ ID NO: 13. Suitably, any of these penton proteins, or unique fragments thereof, may

be utilized for a variety of purposes. Examples of suitable fragments include the penton having N-terminal and/or C-terminal truncations of about 50, 100, 150, or 200 amino acids, based upon the amino acid numbering provided above. Other suitable fragments include shorter internal, C-terminal, or N-terminal fragments. Further, the penton protein may be modified for a variety of purposes known to those of skill in the art.

The invention further provides the amino acid sequences of the hexon protein of SA18, SEQ ID NO:14. Suitably, this hexon protein, or unique fragments thereof, may be utilized for a variety of purposes. Examples of suitable fragments include the hexon having N-terminal and/or C-terminal truncations of about 50, 100, 150, 200, 300, 400, or 500 amino acids, based upon the amino acid numbering provided above and in SEQ ID NO: 14. Other suitable fragments include shorter internal, C-terminal, or N-terminal fragments. For example, one suitable fragment the loop region (domain) of the hexon protein, designated DE1 and FG1, or a hypervariable region thereof. Such fragments include the regions spanning amino acid residues about 125 to 443; about 138 to 441, or smaller fragments, such as those spanning about residue 138 to residue 163; about 170 to about 176; about 195 to about 203; about 233 to about 246; about 253 to about 264; about 287 to about 297; about 404 to about 430, about 430 to 550, about 545 to 650; of the simian hexon proteins, with reference to SEQ ID NO: 14. Other suitable fragments may be readily identified by one of skill in the art. Further, the hexon protein may be modified for a variety of purposes known to those of skill in the art. Because the hexon protein is the determinant for serotype of an adenovirus, such artificial hexon proteins would result in adenoviruses having artificial serotypes. Other artificial capsid proteins can also be constructed using the chimp Ad penton sequences and/or fiber sequences of the invention and/or fragments thereof.

In one example, it may be desirable to generate an adenovirus having an altered hexon protein utilizing the sequences of a hexon protein of the invention. One suitable method for altering hexon proteins is described in US Patent 5,922,315, which is incorporated by reference. In this method, at least one loop region of the adenovirus hexon is changed with at least one loop region of another adenovirus serotype. Thus, at least one loop region of such an altered adenovirus hexon protein is a simian Ad hexon loop region of the invention. In one embodiment, a loop region

of the SA18 hexon protein is replaced by a loop region from another adenovirus serotype. In another embodiment, the loop region of the SA18 hexon is used to replace a loop region from another adenovirus serotype. Suitable adenovirus serotypes may be readily selected from among human and non-human serotypes, as described herein. SA18 is selected for purposes of illustration only; the other simian Ad hexon proteins of the invention may be similarly altered, or used to alter another Ad hexon. The selection of a suitable serotype is not a limitation of the present invention. Still other uses for the hexon protein sequences of the invention will be readily apparent to those of skill in the art.

The invention further encompasses the fiber proteins of the simian adenoviruses of the invention. The fiber protein of AdSA18 has the amino acid sequence of SEQ ID NO: 15. Suitably, this fiber protein, or unique fragments thereof, may be utilized for a variety of purposes. One suitable fragment is the fiber knob, which spans about amino acids 247 to 425 of SEQ ID NO: 15. Examples of other suitable fragments include the fiber having N-terminal and/or C-terminal truncations of about 50, 100, 150, or 200 amino acids, based upon the amino acid numbering provided above and in SEQ ID NO: 15. Still other suitable fragments include internal fragments. Further, the fiber protein may be modified using a variety of techniques known to those of skill in the art.

The invention further encompasses unique fragments of the proteins of the invention which are at least 8 amino acids in length. However, fragments of other desired lengths can be readily utilized. In addition, the invention encompasses such modifications as may be introduced to enhance yield and/or expression of an SA18 gene product, e.g., construction of a fusion molecule in which all or a fragment of the SA18 gene product is fused (either directly or via a linker) with a fusion partner to enhance. Other suitable modifications include, without limitation, truncation of a coding region (e.g., a protein or enzyme) to eliminate a pre- or pro-protein ordinarily cleaved and to provide the mature protein or enzyme and/or mutation of a coding region to provide a secretable gene product. Still other modifications will be readily apparent to one of skill in the art. The invention further encompasses proteins having at least about 95% to 99% identity to the SA18 proteins provided herein.

As described herein, vectors of the invention containing the adenoviral capsid proteins of the invention are particularly well suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other Ad serotype based vectors, as well as other viral vectors. The rAd vectors of the invention are particularly advantageous in readministration for repeat gene therapy or for boosting immune response (vaccine titers). Examples of such regimens are provided herein.

Under certain circumstances, it may be desirable to use one or more of the SA18 gene products (e.g., a capsid protein or a fragment thereof) to generate an antibody. The term "an antibody," as used herein, refers to an immunoglobulin molecule which is able to specifically bind to an epitope. Thus, the antibodies of the invention bind, preferably specifically and without cross-reactivity, to a SA18 epitope. The antibodies in the present invention exist in a variety of forms including, for example, high affinity polyclonal antibodies, monoclonal antibodies, synthetic antibodies, chimeric antibodies, recombinant antibodies and humanized antibodies. Such antibodies originate from immunoglobulin classes IgG, IgM, IgA, IgD and IgE.

Such antibodies may be generated using any of a number of methods know in the art. Suitable antibodies may be generated by well-known conventional techniques, e.g. Kohler and Milstein and the many known modifications thereof. Similarly desirable high titer antibodies are generated by applying known recombinant techniques to the monoclonal or polyclonal antibodies developed to these antigens [see, e.g., PCT Patent Application No. PCT/GB85/00392; British Patent Application Publication No. GB2188638A; Amit *et al.*, 1986 *Science*, *233*:747-753; Queen *et al.*, 1989 *Proc. Nat'l. Acad. Sci. USA*, *86*:10029-10033; PCT Patent Application No. PCT/WO9007861; and Riechmann *et al.*, *Nature*, *332*:323-327 (1988); Huse *et al*, 1988a *Science*, *246*:1275-1281]. Alternatively, antibodies can be produced by manipulating the complementarity determining regions of animal or human antibodies to the antigen of this invention. See, e.g., E. Mark and Padlin, "Humanization of Monoclonal Antibodies", Chapter 4, The Handbook of Experimental Pharmacology, Vol. 113, The Pharmacology of Monoclonal Antibodies, Springer-Verlag (June, 1994); Harlow *et al.*, 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow *et al.*, 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston *et al.*, 1988, *Proc.*

Natl. Acad. Sci. USA 85:5879-5883; and Bird *et al.*, 1988, *Science 242*:423-426. Further provided by the present invention are anti-idiotype antibodies (Ab2) and anti-anti-idiotype antibodies (Ab3). See, e.g., M. Wettendorff et al., "Modulation of anti-tumor immunity by anti-idiotypic antibodies." In Idiotypic Network and Diseases, ed. by J. Cerny and J. Hiernaux, 1990 *J. Am. Soc. Microbiol.*, Washington DC: pp. 203-229]. These anti-idiotype and anti-anti-idiotype antibodies are produced using techniques well known to those of skill in the art. These antibodies may be used for a variety of purposes, including diagnostic and clinical methods and kits.

Under certain circumstances, it may be desirable to introduce a detectable label or a tag onto a SA18 gene product, antibody or other construct of the invention. As used herein, a detectable label is a molecule which is capable, alone or upon interaction with another molecule, of providing a detectable signal. Most desirably, the label is detectable visually, e.g. by fluorescence, for ready use in immunohistochemical analyses or immunofluorescent microscopy. For example, suitable labels include fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), coriphosphine-O (CPO) or tandem dyes, PE-cyanin-5 (PC5), and PE-Texas Red (ECD). All of these fluorescent dyes are commercially available, and their uses known to the art. Other useful labels include a colloidal gold label. Still other useful labels include radioactive compounds or elements. Additionally, labels include a variety of enzyme systems that operate to reveal a colorimetric signal in an assay, e.g., glucose oxidase (which uses glucose as a substrate) releases peroxide as a product which in the presence of peroxidase and a hydrogen donor such as tetramethyl benzidine (TMB) produces an oxidized TMB that is seen as a blue color. Other examples include horseradish peroxidase (HRP) or alkaline phosphatase (AP), and hexokinase in conjunction with glucose-6-phosphate dehydrogenase which reacts with ATP, glucose, and NAD+ to yield, among other products, NADH that is detected as increased absorbance at 340 nm wavelength.

Other label systems that are utilized in the methods of this invention are detectable by other means, e.g., colored latex microparticles [Bangs Laboratories, Indiana] in which a dye is embedded are used in place of enzymes to form conjugates with the target sequences provide a visual signal indicative of the presence of the resulting complex in applicable assays.

Methods for coupling or associating the label with a desired molecule are similarly conventional and known to those of skill in the art. Known methods of label attachment are described [see, for example, Handbook of Fluorescent probes and Research Chemicals, 6th Ed., R. P. M. Haugland, Molecular Probes, Inc., Eugene, OR, 1996; Pierce Catalog and Handbook, Life Science and Analytical Research Products, Pierce Chemical Company, Rockford, IL, 1994/1995]. Thus, selection of the label and coupling methods do not limit this invention.

The sequences, proteins, and fragments of the invention may be produced by any suitable means, including recombinant production, chemical synthesis, or other synthetic means. Suitable production techniques are well known to those of skill in the art. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY). Alternatively, peptides can also be synthesized by the well known solid phase peptide synthesis methods (Merrifield, *J. Am. Chem. Soc.*, 85:2149 (1962); Stewart and Young, Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969) pp. 27-62). These and other suitable production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

In addition, one of skill in the art will readily understand that the Ad sequences of the invention can be readily adapted for use for a variety of viral and non-viral vector systems for *in vitro, ex vivo or in vivo* delivery of therapeutic and immunogenic molecules. For example, in one embodiment, the simian Ad capsid proteins and other simian adenovirus proteins described herein are used for non-viral, protein-based delivery of genes, proteins, and other desirable diagnostic, therapeutic and immunogenic molecules. In one such embodiment, a protein of the invention is linked, directly or indirectly, to a molecule for targeting to cells with a receptor for adenoviruses. Preferably, a capsid protein such as a hexon, penton, fiber or a fragment thereof having a ligand for a cell surface receptor is selected for such targeting. Suitable molecules for delivery are selected from among the therapeutic molecules described herein and their gene products. A variety of linkers including, lipids, polyLys, and the like may be utilized as linkers. For example, the simian penton protein may be readily utilized for such a purpose by production of a fusion protein using the simian penton sequences in a manner analogous to that described in Medina-Kauwe LK, et al, *Gene Ther.* 2001 May; 8(10):795-803 and Medina-Kauwe

LK, et al, *Gene Ther.* 2001 Dec; 8(23): 1753-1761. Alternatively, the amino acid sequences of simian Ad protein IX may be utilized for targeting vectors to a cell surface receptor, as described in US Patent Appln 20010047081. Suitable ligands include a CD40 antigen, an RGD-containing or polylysine-containing sequence, and the like. Still other simian Ad proteins, including, e.g., the hexon protein and/or the fiber protein, may be used for used for these and similar purposes.

Still other adenoviral proteins of the invention may be used as alone, or in combination with other adenoviral protein, for a variety of purposes which will be readily apparent to one of skill in the art. In addition, still other uses for the adenoviral proteins of the invention will be readily apparent to one of skill in the art.

The compositions of this invention include vectors that deliver a heterologous molecule to cells, either for therapeutic or vaccine purposes. Such vectors, containing simian adenovirus DNA of SA18 and a minigene, can be constructed using techniques such as those described herein for the chimeric adenoviruses and such techniques as are known in the art. Alternatively, SA19 may be a source for sequences of the chimeric adenoviruses are described herein.

The following examples are illustrative, and are not intended to limit the invention to those illustrated embodiments.

Example 1 - Construction of Pan5/C1 Chimeric Simian Viruses

Five different adenoviruses initially isolated from the chimpanzee, AdC68 [US Patent 6,083,716], AdPan5, AdPan7, AdPan6 and AdC1 [US Patent 6,083,716] have been sequenced. See, International Application No. PCT/US02/33645, filed November 2002 for the sequences of Pan5 [SEQ ID NO:1], Pan7 [SEQ ID NO:3], and Pan6 [SEQ ID NO:2]. This application also provides sequences for SV1, SV25 and SV39 [SEQ ID No. 4, 5, 6, respectively]. Sequence comparison of the capsid protein sequences predicted that AdC1 clearly belonged to a different serological subgroup than the other four chimpanzee derived adenoviruses.

However, attempts to cultivate AdC1 in HEK293 cells revealed it to be fastidious in its growth characteristics (data not shown) and therefore possibly unsuitable for use as a vector using the currently available E1 complementing cell lines. However, because of the obvious sequence dissimilarity of AdC1 capsid

protein sequence from the other chimpanzee derived adenoviruses (as well as the huAd5), chimeric adenovirus vectors were generated with the capsid characteristics of AdC1. In view of the above-mentioned drawbacks associated with only making hexon changes, more extensive replacements were made in the chimera described herein, i.e., construction of chimeras where the replacement went beyond just the hexon, to achieve two goals. The first was to determine whether making extended replacements would allow for the rescue of viruses containing hexons of unrelated serotypes that may not otherwise be amenable to rescue. The second goal was to test whether the growth characteristics of adenovirus vectors such as AdPan5, that have been found in our laboratory to be able to be grown to high titer for the purpose of manufacture, would also be present in the chimeric virus, particularly when the hexon (and other capsid proteins) are derived from a virus such as AdC1 that are difficult to grow to a high yield in cell lines such as HEK293. An added bonus of extending the replacement to include the fiber protein would be to further increase the antigenic dissimilarity to beyond that afforded by a hexon change alone.

As an alternative to obtaining purified virus as source for adenoviral DNA to sequence, we have resorted to cloning restriction fragments of viral DNA obtained from infected cells ("Hirt prep"). The first adenovirus we have sequenced in this way is Simian Adenovirus. EcoRI digestion of the Simian Adenovirus yielded 7 fragments. Shotgun cloning yielded clones of the 5 internal fragments, which were cloned and sequenced. Completion of the sequencing was carried out by walking towards each of the ends of the genome. The map of the genome is shown in Figure 1.

A. *Construction of Two Pan5/C1 Chimeric Plasmids*

The overall approach towards constructing chimeric viruses was to first assemble the complete E1 deleted virus DNA into a single plasmid flanked by recognition sites for the restriction enzyme SwaI, digest the plasmid DNA with SwaI to release the virus DNA ends, and transfect the DNA into HEK293 cells to determine whether viable chimeric adenovirus could be rescued. Two chimeric virus plasmids were constructed, p5C1short and p5C1long.

The plasmid p5C1short harbors an E1 deleted Pan5 virus where an internal 15226 bp segment (18332 – 33557) has been replaced by a functionally analogous 14127 bp (18531 – 32657) from AdC1. This results in the replacement of

the Pan5 proteins hexon, endoprotease, DNA binding protein, 100 kD scaffolding protein, 33 kD protein, protein VIII, and fiber, as well as the entire E3 region, with the homologous segment from AdC1. The ClaI site at the left end of the AdC1 fragment is at the beginning of the hexon gene and the resulting protein is identical to the C1 hexon. The EcoRI site which constitutes the right end of the AdC1 fragment is within the E4 orf 7 part of the AdC1. The right end was ligated to a PCR generated right end fragment from AdPan5 such that the regenerated orf 7-translation product is chimeric between AdPan5 and AdC1.

The plasmid p5C1long harbors an E1 deleted Pan5 virus where an internal 25603 bp segment (7955 – 33557) has been replaced by a functionally analogous 24712 bp (7946 – 32657) from AdC1. This results in the replacement of the AdPan5 pre-terminal protein, 52/55 kD protein, penton base protein, protein VII, Mu, and protein VI with those from AdC1 in addition to those replaced in p5C1short. The AscI site at the left end of the AdC1 fragment is at the beginning of the DNA polymerase gene and results in a chimeric protein where the first 165 amino acids of the AdPan5 DNA polymerase has been replaced by a 167 amino acid segment from AdC1 DNA polymerase. In this N-terminal region, the homology between the AdPan5 and AdC1 DNA polymerase proteins is 81% (72% identity).

The plasmid pDVP5Mlu which contains the left end of AdPan5 was used as the starting plasmid for the chimeric vector construction.

The plasmid pDVP5Mlu was made as follows. A synthetic DNA fragment harboring recognition sites for the restriction enzymes SmaI, MluI, EcoRI and EcoRV respectively was ligated into pBR322 digested with EcoRI and NdeI so as to retain the origin of replication and the beta-lactamase gene. The left end of Pan5 extending to the MluI site (15135 bp) was cloned into this plasmid between the SmaI and MluI sites. The E1 gene was functionally deleted and replaced by a DNA fragment harboring recognition sites for the extremely rare cutter restriction enzyme sites I-CeuI and PI-SceI). The 2904 base pairs of the right end of Pan-5 was PCR amplified using the primers P5L [GCG CAC GCG TCT CTA TCG ATG AAT TCC ATT GGT GAT GGA CAT GC, SEQ ID NO:7] and P5ITR [GCG CAT TTA AAT CAT CAT CAA TAA TAT ACC TCA AAC, SEQ ID NO:8] using Tgo polymerase (Roche). The PCR product was cut with MluI and SwaI, and cloned between MluI and EcoRV of pDVP5Mlu to yield pPan5Mlu+RE. A 3193 bp fragment extending

from the MluI site (15135) to the ClaI (18328) site of Pan5 was then inserted between the same sites of pPan5Mlu+RE to yield pPan5Cla+RE. The 3671 bp ClaI (18531) to EcoRI (22202) fragment of the adenovirus C1 was cloned into pPan5Cla+RE between ClaI (16111) and EcoRI (16116) to yield pPan5C1delRI. The 10452 bp internal EcoRI fragment of the adenovirus C1 (22202 – 32653) was cloned into the EcoRI site of pPan5C1delRI to yield p5C1short. To construct p5C1long, the AdC1 replacement was further extended by replacing the AscI – ClaI 10379 bp fragment of AdPan5 in p5C1short with the AdC1 AscI – ClaI 10591 bp fragment. Finally a green fluorescent protein (GFP) expression cassette was inserted into both p5C1short and p5C1long between the I-CeuI and PI-SceI sites to yield p5C1shortGFP and p5C1longGFP respectively.

B. *Rescue of chimeric Pan5/C1 recombinant vector adenoviruses*

The plasmids p5C1shortGFP and p5C1longGFP were digested with the restriction enzyme SwaI and transfected into HEK 293 cells. A typical adenovirus induced cytopathic effect was observed. The rescue of recombinant chimeric adenovirus from the p5C1longGFP transfection was confirmed by collecting the supernatant from the transfection and re-infecting fresh cells which were found to be transduced as determined by GFP expression. Viral DNA prepared from the chimeric recombinant virus was digested with several restriction enzymes and found to have the expected pattern on electrophoresis (data not shown).

The chimeric adenoviral construct with the shorter replacement p5C1short encodes the C1 proteins hexon and fiber as well as the intervening open reading frames for endoprotease, DNA binding protein, 100 kDa scaffolding protein, 33 kDa protein, and protein VIII. (The E3 region is also included within this region but is unlikely to impact on the viability of the chimeric virus). When the replacement was extended to include the additional AdC1 proteins pTP (pre-terminal protein), 52/55 kDa protein, penton base, protein VII, Mu, and protein VI, there was no difficulty in rescuing viable chimeric virus. In this experiment, the chimeric adenovirus construction strategy utilized the presence of AscI and ClaI restriction enzyme sites present on the genes for DNA polymerase and hexon respectively on both AdPan5 and AdC1.

The reasons for the relatively higher yield of the chimeric virus compared to the wild–type AdC1 virus are not clear. In the growth of the 5C1

chimeric virus in 293 cells, the adenoviral early region gene products of E1 and E4 are derived from Ad5 and AdPan5 respectively. The E1 and E4 gene products bind, regulate and de-repress several cellular transcription complexes and coordinate their activity towards viral multiplication. Thus it is possible that the E1 gene products supplied in *trans* from the 293 cells and the E4 gene products from AdPan5 are more optimal in the human 293 cell background than are the equivalent AdC1 gene products. This may also apply to the activity of the major late promoter whose activity is responsible for the transcription of the capsid protein genes. In the chimeric virus, the major late promoter, and the protein IVa2 which transactivates it, are derived from AdPan5. However the E2 gene products required for adenoviral DNA replication pTP and single-stranded DNA – binding protein are derived from AdC1. The adenoviral DNA polymerase, which complexes with pTP, is chimeric in Ad5C1 but mostly AdPan5 derived.

Two plasmids have been constructed where the structural proteins derive from the chimpanzee adenovirus Pan 7 and Simian adenovirus 25 respectively, and the flanking sequences are derived from human Ad5 (the commonly used vector strain). The Adhu5-Pan7 chimeric adenovirus has been rescued, demonstrating that the chimeric virus construction method used to derive the Pan 5 – C1 chimeric virus is broadly applicable. Fig. 2 provides the map of the recombinant Adhu5-SV25 chimeric virus. The portion of the genome replaced by DNA from Pan7 is indicated.

Example 2 – C5C1 chimeric vector of invention as a delivery vehicle for immunogenic compositions

A Pan 5 (Simian adenovirus 22, a subgroup E adenovirus) - C1 (Simian adenovirus 21, a subgroup B adenovirus) chimeric expressing the Ebola virus (Zaire) glycoprotein (C5C1-CMVGP) was constructed as a model antigen in order to test the efficacy of the vector C5C1-CMVGP as a vaccine; this vector has been compared it to the Adhu5 based vector (H5-CMVGP). Compared to H5-CMVGP, the C5C1-CMVGP vector yielded only a slightly decreased level of GP expression in transduced A549 cells.

Thereafter, GP-specific T cell and B cell responses elicited in B10BR mice vaccinated with either H5-CMVGP or C5C1-CMVGP vectors were compared. The

C5C1-CMVGP vector appeared to induce lower frequencies of gamma interferon producing CD8+ T cells with kinetics slower than the H5-CMVGP vector as determined by intracellular cytokine staining using a H-2k restricted GP-specific peptide as stimulant. The total IgG response to GP, measured by ELISA, was equivalent in serum from mice vaccinated with the C5C1-CMVGP or the H5-CMVGP vectors. However, the C5C1-CMVGP vector induced a more potent Th1 type response while the H5-CMVGP vector stimulated a more balanced Th1/Th2 type response.

Example 3 – Generation of Simian Pan5/Human Ad40 Chimeric Adenovirus and Chimpanzee Pan5/Simian SA18 Chimeric Adenovirus

The construction of plasmids designed to rescue chimeric adenoviruses where the outside flanking regions are derived from the chimpanzee adenovirus AdPan5, and the internal region (containing the structural capsid protein genes) are derived from the human adenovirus Ad40 and the simian adenovirus SA18, are described below.

As described for the Pan5-C1 chimeric adenovirus, the overall approach towards constructing chimeric viruses was to first assemble the complete E1 deleted virus DNA into a single plasmid flanked by recognition sites for the restriction enzyme SwaI, digest the plasmid DNA with SwaI to release the virus DNA ends, and transfect the DNA into HEK293 cells to determine whether viable chimeric adenovirus could be rescued. Two chimeric virus plasmids were constructed, pPan5-40 and pPan5-SA18 corresponding to the two chimeric adenoviruses referred to above. The plasmid pPan5-40 harbors an E1 deleted Pan5 virus where an internal 22975 bp segment (10400 – 33374) has been replaced by a functionally analogous 21603 bp (10043 – 21603) from Ad40. This results in the replacement of the AdPan5 52/55 kD protein, penton base protein, protein VII, Mu, protein VI, hexon, endoprotease, DNA binding protein, 100 kD scaffolding protein, 33 kD protein, protein VIII, and fiber, as well as the entire E3 region, with the homologous segment from Ad40. Similarly, the plasmid pPan5-SA18 harbors an E1 deleted Pan5 virus where an internal 22975 bp segment (10400 – 33374) has been replaced by a functionally analogous 19015 bp (10573 – 29587) from SA18. This results in the replacement of the AdPan5 52/55 kD protein, penton base protein, protein VII, Mu,

protein VI, hexon, endoprotease, DNA binding protein, 100 kD scaffolding protein, 33 kD protein, protein VIII, and fiber, as well as the entire E3 region, with the homologous segment from SA18.

The construction of plasmids designed to rescue chimeric adenoviruses where the outside flanking regions are derived from the chimpanzee adenovirus AdPan5, and the internal region (containing the structural capsid protein genes) are derived from the human adenovirus Ad40 and the simian adenovirus SA18, are described below.

A. *Silent mutagenesis of XbaI site:*

The plasmid pDVP5Mlu which contains the left end of AdPan5 was used as the starting plasmid for the chimeric vector construction. As a first step the XbaI site (3820) was mutagenized to destroy the recognition site without changing the coding sequence for polymerase. This was done by first sub-cloning the NdeI (812) to HindIII (4931) fragment into the plasmid pNEB193 (New England Biolabs) using the same restriction sites in pNEB193, to yield pNEBp5. A PCR reaction was performed on pNEBp5 using the primers P5XTOP (GATACCTAGGAACGAGGAGGATTTGATATTG, SEQ ID NO:9) and P5XBOT (ATGTACGCCTCCGCGCTCAC, SEQ ID NO:10) to yield a 591 bp product. The PCR product was cleaved with AvrII and BbvCI and ligated into pNEBp5 cut with XbaI and BbvCI to yield the desired mutation in the plasmid pNEBp5mut. The mutated NdeI-HindIII fragment from pNEBp5mut was ligated back into pDVP5Mlu to yield the desired mutated plasmid pDVP5Mlumut.

B. *Insertion of the Pan5 right end comprising the right ITR and the complete E4 region:*

The right end of Pan-5 was PCR amplified (P5RE2PCR) using the primers P5E4 [GATCGAATTCCCACTCTGTACCCCATCTCTG, SEQ ID NO:11] and P5ITR [GCG CAT TTA AAT CAT CAT CAA TAA TAT ACC TCA AAC, SEQ ID NO:8] using Tgo polymerase, cut with EcoRI and SwaI, and cloned between EcoRI and EcoRV of pDVP5Mlumut to yield pPan5Mlumut+RE.

C. *Insertion of Ad40 or SA18 structural protein sequences:*

In order to construct p5-40, the Ad40 segment from XbaI (10038) to EcoRI (31642) was ligated into pDVP5Mlumut+RE2 between XbaI (8178) and

EcoRI (12924) in two steps: first, the XbaI (30494) to EcoRI (31642) fragment was inserted, followed by the XbaI (10038) – XbaI (30494) fragment.
To construct pPan5-SA18 the XbaI (10568) to EcoRI (29584) fragment from the simian adenovirus SA18 was inserted into pDVP5Mlumut+RE2 between XbaI (8178) and EcoRI (12924).

The minigene encoding for green fluorescent protein was inserted in place of the E1 deletion between the I-CeuI and PI-SceI sites in pPan5-40 and pPan5-SA18 respectively. This plasmids were purified, digested with SwaI and transfected into 293 cells.

EXAMPLE 4 – Generation of Chimeric Pan6/Pan7 Vectors

A panel of GFP expressing vectors were generated. This panel includes vectors that are chimeric between Pan 6 and Pan 7 where the hexon protein or the fiber protein or both hexon and fiber proteins of the Pan 7 vector have been replaced by those from Pan 6. Balb/C mice were immunized with either Pan 6 or Pan 7 and re-administration (by tail vein injection) was attempted 3 weeks later using each of the five GFP expressing vectors (C6-GFP, C7-GFP, and the three chimeric vectors). Three days later the level of liver transduction was estimated qualitatively by examining liver sections for the presence of GFP expression and quantitatively by estimating copies of GFP DNA by Taqman analysis. The data showed that antibodies to both hexon and fiber are important in preventing re-administration of adenoviral vectors.

All publications cited in this specification are incorporated herein by reference. While the invention has been described with reference to a particularly preferred embodiment, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A method of efficiently culturing a chimeric adenovirus in a selected host cell, said chimeric adenovirus being from a parental adenovirus strain incapable of efficient growth in said host cell, said method comprising the steps of:

(a) generating a chimeric adenovirus comprising:

(i) adenovirus sequences of the left terminal end and right terminal end of a first adenovirus which grows in a selected host cell type, said left end region comprising the 5' inverted terminal repeat (ITRs), and said right end region comprising the 3' inverted terminal repeat (ITRs); and

(ii) the internal regions from a parental adenovirus which lacks its native 5' and 3' terminal regions, said internal regions comprising the late genes encoding the penton, hexon, and fiber;

wherein the resulting chimeric adenovirus comprises, from 5' to 3', a left terminal region of the first adenovirus, the internal region of the parental adenovirus, and the right terminal region of the first adenovirus; and

b) culturing said chimeric adenovirus in the presence of functional adenovirus E1a, E1b, and E4 ORF6 genes from the first adenovirus or from an adenovirus serotype which transcomplements the first adenovirus, and further in the presence of necessary adenoviral structural genes from the left end of the adenovirus.

2. The method according to claim 1, wherein the internal region of the parental adenovirus further comprises one or more functional adenovirus genes selected from the group consisting of Endoprotease open reading frame, DNA binding protein, 100 kDa scaffolding protein, 33 kDa protein, protein VIII, pTP, 52/55 kDa protein, protein VII, Mu and protein VI.

3. The method according to claim 1, wherein the polymerase, terminal protein and 52/55 kDa protein functions are provided in *trans*.

4. The method according to claim 1, wherein the first adenovirus further comprises the polymerase, terminal protein and 52/55 kDa protein functions.

5. The method according to claim 1, wherein the chimeric adenovirus comprises the adenoviral late genes 1, 2, 3, 4, and 5 of the parental adenovirus.

6. The method according to claim 1, wherein the selected host cell stably contains one or more of the adenovirus E1a, E1b or E4 ORF6 functions.

7. The method according to claim 1, wherein the chimeric adenovirus comprises one or more of the adenovirus E1a, E1b or E4 ORF6 of the first adenovirus.

8. The method according to claim 1, wherein the first adenovirus is of human origin.

9. The method according to claim 1, wherein the first adenovirus is of simian origin.

10. The method according to claim 1, further comprising the step of isolating the chimeric adenovirus.

11. A method for generating a chimeric adenovirus for growth in a selected host cell, said chimeric adenovirus being derived from a parental adenovirus strain incapable of efficient growth in said host cell, said method comprising the step of generating a chimeric adenovirus comprising:

5' and 3' terminal regions of a first adenovirus which grows in a selected host cell type, said 5' terminal regions comprising the 5' inverted terminal repeat (ITRs) and necessary E1 gene functions, and said 3' terminal regions comprising inverted terminal repeat (ITRs) and necessary E4 gene functions; and

internal regions from a parental adenovirus which lacks its native 5' and 3' terminal regions, said internal regions comprising the hexon, penton base and fiber;

wherein the resulting chimeric adenovirus comprises, from 5' to 3', the 5' terminal region of the first adenovirus, the internal region of the parental adenovirus, and the 3' terminal regions of the first adenovirus.

12. A chimeric adenovirus produced according to the method of claim 1.

13. A chimeric adenovirus comprising a hexon protein of a selected adenovirus serotype which is incapable of efficient growth in a selected host cell, said modified adenovirus comprising:

(a) adenovirus sequences of the left terminal end of a first adenovirus which grows in a selected host cell type, said left end region comprising the E1a, E1b and 5' inverted terminal repeat (ITRs);

(b) adenovirus sequences of the internal region of the selected adenovirus serotype which is incapable of efficient growth in the selected host cell, said internal region comprising the genes encoding the penton, hexon and fiber of the selected adenovirus;

(c) adenovirus sequences of the right terminal end of the first adenovirus, said right end region comprising the necessary E4 gene functions and the 3' inverted terminal repeat (ITRs),

wherein the resulting chimeric adenovirus comprises adenoviral structural and regulatory proteins necessary for infection and replication.

14. The chimeric adenovirus according to claim 13, wherein the chimeric adenovirus further comprises the IIIa, 52/55kDa and terminal protein (pTP) of the selected adenovirus serotype.

15. The chimeric adenovirus according to claim 13, wherein chimeric adenovirus comprises the polymerase of the first adenovirus.

16. The chimeric adenovirus according to claim 13, wherein the chimeric adenovirus expresses a functional chimeric protein formed from the first adenovirus and the selected adenovirus, said chimeric protein is selected from the group consisting of polymerase, terminal protein, 52/55 kDa protein, and IIIa.

17. The chimeric adenovirus according to claim 13, wherein the chimeric adenovirus comprises the terminal protein, 52/55 kDa, and/or IIIa of the selected adenovirus.

18. A host cell comprising a chimeric adenovirus according to claim 12.

19. The host cell according to claim 18, wherein said host cell is a human cell.

20. An isolated simian adenovirus nucleic acid sequence selected from the group consisting of:

(a) SA18 having the sequence of nucleic acids 1 to 31967 of SEQ ID NO:12 and

(b) a nucleic acid sequence complementary to the sequence of any of (a) to (f).

21. An isolated simian adenovirus serotype nucleic acid sequence selected from one or more of the group consisting of:

(a) 5' inverted terminal repeat (ITR) sequences;

(b) the adenovirus E1a region, or a fragment thereof selected from among the 13S, 12S and 9S regions;

(c) the adenovirus E1b region, or a fragment thereof selected from among the group consisting of the small T, large T, IX, and IVa2 regions;

(d) the E2b region;

(e) the L1 region, or a fragment thereof selected from among the group consisting of the 28.1 kD protein, polymerase, agnoprotein, 52/55 kD protein, and IIIa protein;

(f) the L2 region, or a fragment thereof selected from the group consisting of the penton, VII, VI, and Mu proteins;

(g) the L3 region, or a fragment thereof selected from the group consisting of the VI, hexon, or endoprotease;

(h) the 2a protein;

(i) the L4 region, or a fragment thereof selected from the group consisting of the 100 kD protein, the 33 kD homolog, and VIII;

(j) the E3 region, or a fragment thereof selected from the group consisting of E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) the L5 region, or a fragment thereof selected from a fiber protein;

(l) the E4 region, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1;and

(m) the 3' ITR, of any of SA18 SEQ ID NO:12, or a sequence complementary to any of (a) to (m).

22. A simian adenovirus protein encoded by the nucleic acid sequence according to claim 21.

23. A composition comprising a simian adenovirus capsid protein according to claim 22 linked to a heterologous molecule for delivery to a selected host cell.

24. A method for targeting a cell having an adenoviral receptor comprising delivering to a subject a composition according to claim 23.

25. A nucleic acid molecule comprising a heterologous simian adenoviral sequence according to claim 21.

26. The nucleic acid molecule according to claim 25, wherein said simian adenoviral sequence encodes an adenoviral gene product and is operatively linked to regulatory control sequences which direct expression of the adenoviral gene product in a host cells.

27. The nucleic acid molecule according to claim 25, wherein said simian adenoviral sequence comprises the E1a region of SA18 SEQ ID NO:12.

28. A pharmaceutical composition comprising the nucleic acid molecule according to claim 27 and a physiologically compatible carrier.

29. A recombinant adenovirus having a capsid comprising a protein selected from the group consisting of:

(a) a hexon protein of SA18, SEQ ID NO 13, or a unique fragment thereof;

(b) a penton protein of SA18, SEQ ID NO: 14, or a unique fragment thereof;

(c) a fiber protein of SA18, SEQ ID NO: 15, or a unique fragment thereof.

30. The recombinant adenovirus according to claim 29, wherein the capsid is of an artificial serotype.

31. The recombinant adenovirus according to claim 29, wherein said virus further comprises a heterologous gene operatively linked to sequences which direct expression of said gene in a host cell.

32. The recombinant adenovirus according to claim 29, further comprising 5' and 3' adenovirus cis-elements necessary for replication and encapsidation.

33. The recombinant adenovirus according to claim 29, wherein said vector lacks all or a part of the E1 gene.

34. A host cell comprising a heterologous nucleic acid molecule comprising the nucleic acid sequence according to claim 21.

35. The host cell according to claim 34, wherein said host cell is stably transformed with the nucleic acid molecule.

36. The host cell according to claim 34, wherein said host cell expresses one or more adenoviral gene products from said nucleic acid molecule, said adenoviral gene products selected from the group consisting of E1a, E1b, E2a, and E4 ORF6.

37. The host cell according to claim 34, wherein said host cell is stably transformed with a nucleic acid molecule comprising the simian adenovirus inverted terminal repeats.

38. A composition comprising a recombinant virus according to claim 29 in a pharmaceutically acceptable carrier.

39. A method for delivering a heterologous gene to a mammalian cell comprising introducing into said cell an effective amount of the recombinant virus according to claim 29.

40. A method for repeat administration of a heterologous gene to a mammal comprising the steps of:

(a) introducing into said mammal a first vector which comprises the heterologous gene and

(b) introducing into said mammal a second vector which comprises the heterologous gene;

wherein at least the first virus or the second vector is a virus according to claim 29 and wherein the first and second recombinant vector are different.

41. A method for producing a selected gene product comprising infecting a mammalian cell with the recombinant virus according to claim 29, culturing said cell under suitable conditions and recovering from said cell culture the expressed gene product.

42. A method for eliciting an immune response in a mammalian host against an infective agent comprising administering to said host an effective amount of the

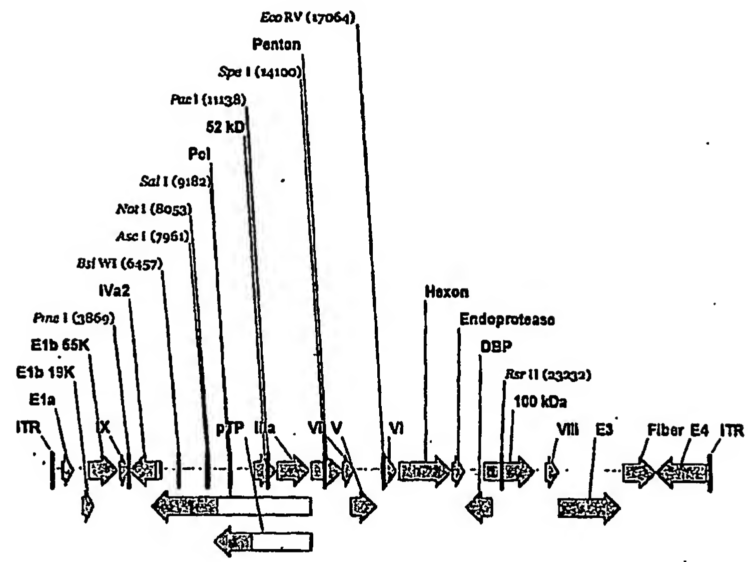
recombinant adenovirus of claim 29, wherein said heterologous gene encodes an antigen of the infective agent.

43. The method according to claim 42, comprising the step of priming the host with a DNA vaccine comprising the heterologous gene prior to administering the recombinant adenovirus.

ABSTRACT OF THE DISCLOSURE

A method for providing an adenovirus from a serotype which does not grow efficiently in a desired cell line with the ability to grow in that cell line is described. The method involves replacing the left and right termini of the adenovirus with the corresponding termini from an adenovirus which grow efficiently in the desired cell line. At a minimum, the left terminus spans the 5' inverted terminal repeat, the left terminus spans the E4 region and the 3' inverted terminal repeat. The resulting chimeric adenovirus contains the internal regions spanning the genes encoding the penton, hexon and fiber from the serotype which does not grow efficiently in the desired cell. Also provided are vectors constructed from novel simian adenovirus sequences and proteins, host cells containing same, and uses thereof..

Fig. 1



Simian Adenovirus
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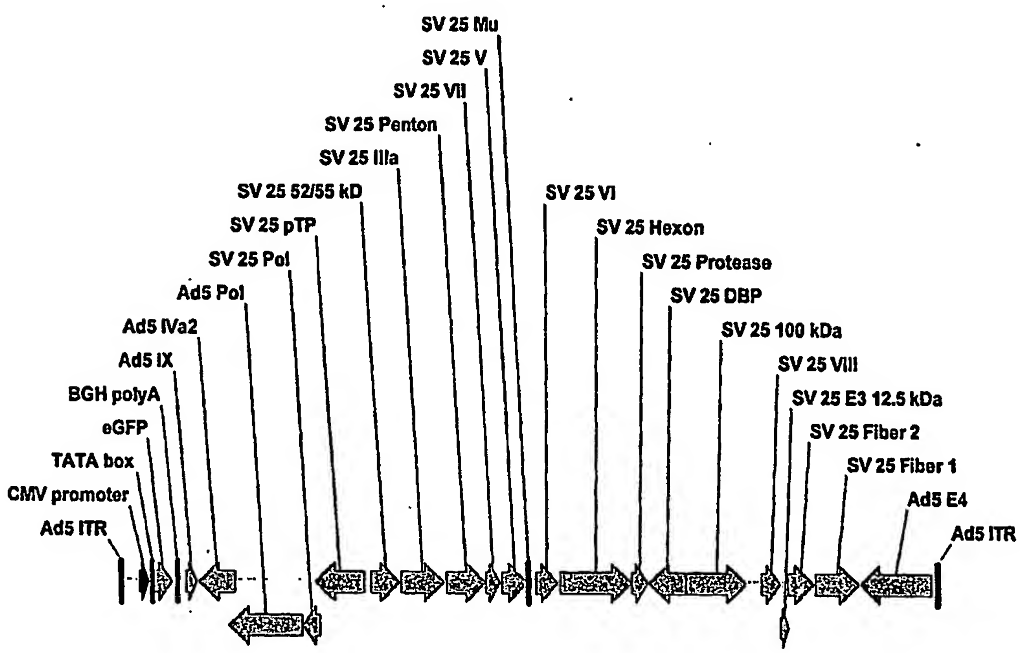
Fig. 2



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SEQUENCE LISTING

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Roy, Soumitra

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Tyr Glu Trp Asn Phe Arg Lys Asp Val Asn Met Ile Leu Gln Ser
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Ser Leu Gly Asn Asp Leu Arg Val Asp Gly Ala Ser Val Arg Phe
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Asp Ser Ile Asn Leu Tyr Ala Asn Phe Phe Pro Met Ala His Asn
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Thr Ala Ser Thr Leu Glu Ala Met Leu Arg Asn Asp Thr Asn Asp
1100 1105 1110

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Gln Ser Phe Asn Asp Tyr Leu Cys Ala Ala Asn Met Leu Tyr Pro
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Ile Pro Ala Asn Ala Thr Ser Val Pro Ile Ser Ile Pro Ser Arg
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Asn Trp Ala Ala Phe Arg Gly Trp Ser Phe Thr Arg Leu Lys Thr
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Lys Glu Thr Pro Ser Leu Gly Ser Gly Phe Asp Pro Tyr Phe Thr
1160 1165 1170

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Tyr Ser Gly Ser Ile Pro Tyr Leu Asp Gly Thr Phe Tyr Leu Asn
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His Thr Phe Lys Lys Val Ser Ile Met Phe Asp Ser Ser Val Ser
1190 1195 1200

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Lys Arg Thr Val Asp Gly Glu Gly Tyr Asn Val Ala Gln Cys Asn
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Met Thr Lys Asp Trp Phe Leu Ile Gln Met Leu Ser His Tyr Asn
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Ile Gly Tyr Gln Gly Phe Tyr Val Pro Glu Gly Tyr Lys Asp Arg
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Met Tyr Ser Phe Phe Arg Asn Phe Gln Pro Met Ser Arg Gln Val
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Val Asp Thr Thr Thr Tyr Thr Asp Tyr Lys Asn Val Thr Leu Pro
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Phe Gln His Asn Asn Ser Gly Phe Val Gly Tyr Met Gly Pro Thr
1295 1300 1305

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Met Arg Glu Gly Gln Ala Tyr Pro Ala Asn Tyr Pro Tyr Pro Leu
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Ile Gly Lys Thr Ala Val Pro Ser Leu Thr Gln Lys Lys Phe Leu
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Cys Asp Arg Thr Met Trp Arg Ile Pro Phe Ser Ser Asn Phe Met
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Ser Met Gly Ala Leu Thr Asp Leu Gly Gln Asn Met Leu Tyr Ala
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Asp Glu Pro Thr Leu Leu Tyr Val Leu Phe Glu Val Phe Asp Val
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Val Arg Ile His Gln Pro His Arg Gly Val Ile Glu Ala Val Tyr
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Leu Arg Thr Pro Phe Ser Ala Gly Asn Ala Thr Thr
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Met Lys

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Arg Ala Arg Leu Asp Asp Asp Phe Asn Pro Val Tyr Pro Tyr Asp
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Thr Pro Asn Ala Pro Ser Val Pro Phe Ile Thr Pro Pro Phe Val
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Lys Leu Gly Ser Gly Leu Asn Ile Asn Gln Asp Gly Glu Leu Thr
1490 1495 1500

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Ser Asp Ala Ser Val Leu Val Thr Pro Pro Ile Thr Lys Ala Asn

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Asn Thr Ile Gly Leu Ala Phe Asn Ala Pro Leu Thr Leu Gln Ser
1520 1525 1530

gat act tta aat ctt gct tgt aac gcc cca ctt acc gtg caa gac 27557
Asp Thr Leu Asn Leu Ala Cys Asn Ala Pro Leu Thr Val Gln Asp
1535 1540 1545

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Ser Glu Leu Ala Leu Ala Val Thr Pro Pro Leu Asp Thr Ala Asn
1565 1570 1575

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Asn Thr Leu Ala Leu Lys Thr Ala Arg Pro Ile Ile Thr Asn Ser
1580 1585 1590

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1595 1600 1605

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Thr Thr Gly Ser Met Ala Met Gln Ile Ser Gln Pro Leu Lys Val
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Glu Asp Gly Ser Leu Ser Leu Ser Ile Glu Ser Pro Leu Asn Leu
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Gln Asn Asn Ala Leu Ala Leu Ser Val Leu Leu Pro Leu Arg Leu
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1790 1795 1800

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Leu Arg Tyr Asn Gly Thr Tyr Lys Asn Leu Ala Val Lys Thr Asp
1805 1810 1815

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Ser Phe Arg Gly Leu Glu Ile Asp Ser Asn Gln Phe Leu Val Pro
1820 1825 1830

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Arg Leu Gly Ser Gly Leu Lys Phe Asp Gln Tyr Gly Tyr Ile Ser
1835 1840 1845

gtc ata cct cca act gtt acg cca aca aca ctt tgg act aca gca 28502
Val Ile Pro Pro Thr Val Thr Pro Thr Thr Leu Trp Thr Thr Ala
1850 1855 1860

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Asp Pro Ser Pro Asn Ala Thr Phe Tyr Asp Ser Leu Asp Ala Lys
1865 1870 1875

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Val Trp Leu Ala Leu Val Lys Cys Asn Gly Met Val Asn Gly Thr
1880 1885 1890

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Ile Ala Ile Lys Ala Leu Lys Gly Thr Leu Leu Gln Pro Thr Ala
1895 1900 1905

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Ser Phe Ile Ser Phe Val Met Tyr Phe Tyr Ser Asn Gly Thr Arg
1910 1915 1920

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Arg Thr Asn Tyr Pro Thr Phe Glu Asn Glu Gly Ile Leu Ala Ser
1925 1930 1935

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Ser Ala Thr Trp Gly Tyr Arg Gln Gly Asn Ser Ala Asn Thr Asn
1940 1945 1950

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Val Thr Ser Ala Val Glu Phe Met Pro Ser Ser Thr Arg Tyr Pro
1955 1960 1965

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Phe Leu Gln Gly Asp Pro Thr Met Ala Ile Ser Phe Gln Ala Ile
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Tyr Asn His Ala Leu Glu Gly Tyr Ser Leu Lys Phe Thr Trp Arg
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2015 2020 2025

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caccegcccc gaacaaactc caaaagtcca aaaagtccaa aacgcccgcg taaaagcccg 31745

ccaaaacagc acttcctcat ttactctccc acagtacgtc acttccgccg cgcccgccgc 31805

cctcgccccg ccctcaccct cgcgctccac cccgcgcccc acgtcagact cccaccccgc 31865

cccgcgcccg cgtcatccgc accccaccct cactccaccc ctaaccccgc ctcctcatta 31925

tcatattggc accgttccca aataaggtat attatgatga tg 31967

<210> 13
<211> 508
<212> PRT
<213> Simian adenovirus

<400> 13

Met Arg Arg Ala Val Gly Val Pro Pro Val Met Ala Tyr Ala Glu Gly
1 5 10 15

Pro Pro Pro Ser Tyr Glu Thr Val Met Gly Ala Ala Asp Ser Pro Ala
20 25 30

Thr Leu Glu Ala Leu Tyr Val Pro Pro Arg Tyr Leu Gly Pro Thr Glu
35 40 45

Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Tyr Asp Thr
50 55 60

Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Ala Asp Ile Ala Ser Leu
65 70 75 80

Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Val Gln Asn
85 90 95

Asn Asp Phe Thr Pro Val Glu Ala Gly Thr Gln Thr Ile Asn Phe Asp
100 105 110

Glu Arg Ser Arg Trp Gly Gly Asp Leu Lys Thr Ile Leu Arg Thr Asn
115 120 125

Met Pro Asn Ile Asn Glu Phe Met Ser Thr Asn Lys Phe Arg Ala Arg
130 135 140

Leu Met Val Glu Lys Val Asn Lys Glu Thr Asn Ala Pro Arg Tyr Glu

145   150   155   160

Trp Phe Glu Phe Thr Leu Pro Glu Gly Asn Tyr Ser Glu Thr Met Thr
            165   170   175

Ile Asp Leu Met Asn Asn Ala Ile Val Asp Asn Tyr Leu Glu Val Gly
        180   185   190

Arg Gln Asn Gly Val Leu Glu Ser Asp Ile Gly Val Lys Phe Asp Thr
    195   200   205

Arg Asn Phe Arg Leu Gly Trp Asp Pro Val Thr Lys Leu Val Met Pro
210   215   220

Gly Val Tyr Thr Asn Glu Ala Phe His Pro Asp Ile Val Leu Leu Pro
225   230   235   240

Gly Cys Gly Val Asp Phe Thr Gln Ser Arg Leu Ser Asn Leu Leu Gly
            245   250   255

Ile Arg Lys Arg Met Pro Phe Gln Ala Gly Phe Gln Ile Met Tyr Glu
        260   265   270

Asp Leu Glu Gly Gly Asn Ile Pro Ala Leu Leu Asp Val Ala Lys Tyr
    275   280   285

Glu Ala Ser Ile Gln Lys Ala Arg Glu Gln Gly Gln Glu Ile Arg Gly
290   295   300

Asp Asn Phe Thr Val Ile Pro Arg Asp Val Glu Ile Val Pro Val Glu
305   310   315   320

Lys Asp Ser Lys Asp Arg Ser Tyr Asn Leu Leu Pro Gly Asp Gln Thr
            325   330   335

Asn Thr Ala Tyr Arg Ser Trp Phe Leu Ala Tyr Asn Tyr Gly Asp Pro
        340   345   350

Glu Lys Gly Val Arg Ser Trp Thr Leu Leu Thr Thr Thr Asp Val Thr
    355   360   365

Cys Gly Ser Gln Gln Val Tyr Trp Ser Leu Pro Asp Met Met Gln Asp
370   375   380

Pro Val Thr Phe Arg Pro Ser Ser Gln Val Ser Asn Tyr Pro Val Val
385   390   395   400

Gly Val Glu Leu Leu Pro Val His Ala Lys Ser Phe Tyr Asn Glu Gln
                405   410   415

Ala Val Tyr Ser Gln Leu Ile Arg Gln Ser Thr Ala Leu Thr His Val
            420   425   430

Phe Asn Arg Phe Pro Glu Asn Gln Ile Leu Val Arg Pro Pro Ala Pro
        435   440   445

Thr Ile Thr Thr Val Ser Glu Asn Val Pro Ala Leu Thr Asp His Gly
    450   455   460

Thr Leu Pro Leu Arg Ser Ser Ile Ser Gly Val Gln Arg Val Thr Ile
465   470   475   480

Thr Asp Ala Arg Arg Arg Thr Cys Pro Tyr Val His Lys Ala Leu Gly
                485   490   495

Ile Val Ala Pro Lys Val Leu Ser Ser Arg Thr Phe
            500   505

<210> 14
<211> 917
<212> PRT
<213> Simian adenovirus

<400> 14

Met Ala Thr Pro Ser Met Met Pro Gln Trp Ser Tyr Met His Ile Ala
1   5   10   15

Gly Gln Asp Ala Ser Glu Tyr Leu Ser Pro Gly Leu Val Gln Phe Ala
            20   25   30

Arg Ala Thr Asp Thr Tyr Phe Ser Leu Gly Asn Lys Phe Arg Asn Pro
        35   40   45

Thr Val Ala Pro Thr His Asp Val Thr Thr Asp Arg Ser Gln Arg Leu
    50   55   60

Thr Leu Arg Phe Val Pro Val Asp Arg Glu Asp Thr Ala Tyr Ser Tyr
65 70 75 80

Lys Val Arg Phe Thr Leu Ala Val Gly Asp Asn Arg Val Leu Asp Met
85 90 95

Ala Ser Thr Tyr Phe Asp Ile Arg Gly Val Leu Asp Arg Gly Pro Ser
100 105 110

Phe Lys Pro Tyr Ser Gly Thr Ala Tyr Asn Ser Leu Ala Pro Lys Gly
115 120 125

Ala Pro Asn Pro Ser Glu Trp Lys Gly Ser Asp Asn Lys Ile Ser Val
130 135 140

Arg Gly Gln Ala Pro Phe Phe Ser Thr Ser Ile Thr Lys Asp Gly Ile
145 150 155 160

Gln Val Ala Thr Asp Thr Ser Ser Gly Ala Val Tyr Ala Lys Lys Glu
165 170 175

Tyr Gln Pro Glu Pro Gln Val Gly Gln Glu Gln Trp Asn Ser Glu Ala
180 185 190

Ser Asp Ser Asp Lys Val Ala Gly Arg Ile Leu Lys Asp Thr Thr Pro
195 200 205

Met Phe Pro Cys Tyr Gly Ser Tyr Ala Lys Pro Thr Asn Glu Gln Gly
210 215 220

Gly Gln Gly Thr Asn Thr Val Asp Leu Gln Phe Phe Ala Ser Ser Ser
225 230 235 240

Ala Thr Ser Thr Pro Lys Ala Val Leu Tyr Ala Glu Asp Val Ala Ile
245 250 255

Glu Ala Pro Asp Thr His Leu Val Tyr Lys Pro Ala Val Thr Thr Thr
260 265 270

Thr Thr Ser Ser Gln Asp Leu Leu Thr Gln Gln Ala Ala Pro Asn Arg
275 280 285

Pro Asn Tyr Ile Gly Phe Arg Asp Asn Phe Ile Gly Leu Met Tyr Tyr

290 295 300

Asn Ser Thr Gly Asn Met Gly Val Leu Ala Gly Gln Ala Ser Gln Leu
305 310 315 320

Asn Ala Val Val Asp Leu Gln Asp Arg Asn Thr Glu Leu Ser Tyr Gln
325 330 335

Leu Met Leu Asp Ala Leu Gly Asp Arg Ser Arg Tyr Phe Ser Met Trp
340 345 350

Asn Gln Ala Val Asp Ser Tyr Asp Pro Asp Val Arg Ile Ile Glu Asn
355 360 365

His Gly Val Glu Asp Glu Leu Pro Asn Tyr Cys Phe Pro Leu Gly Gly
370 375 380

Ser Leu Val Thr Glu Thr Tyr Thr Gly Leu Ser Pro Gln Asn Gly Ser
385 390 395 400

Asn Thr Trp Thr Thr Asp Ser Thr Thr Tyr Ala Thr Arg Gly Val Glu
405 410 415

Ile Gly Ser Gly Asn Met Phe Ala Met Glu Ile Asn Leu Ala Ala Asn
420 425 430

Leu Trp Arg Ser Phe Leu Tyr Ser Asn Val Ala Leu Tyr Leu Pro Asp
435 440 445

Glu Tyr Lys Leu Thr Pro Asp Asn Ile Thr Leu Pro Asp Asn Lys Asn
450 455 460

Thr Tyr Asp Tyr Met Asn Gly Arg Val Ala Ala Pro Ser Ser Leu Asp
465 470 475 480

Thr Tyr Val Asn Ile Gly Ala Arg Trp Ser Pro Asp Pro Met Asp Asn
485 490 495

Val Asn Pro Phe Asn His His Arg Asn Ala Gly Leu Arg Tyr Arg Ser
500 505 510

Met Leu Leu Gly Asn Gly Arg Tyr Val Pro Phe His Ile Gln Val Pro
515 520 525

Gln Lys Phe Phe Ala Ile Lys Asn Leu Leu Leu Leu Pro Gly Ser Tyr
530 535 540

Thr Tyr Glu Trp Asn Phe Arg Lys Asp Val Asn Met Ile Leu Gln Ser
545 550 555 560

Ser Leu Gly Asn Asp Leu Arg Val Asp Gly Ala Ser Val Arg Phe Asp
565 570 575

Ser Ile Asn Leu Tyr Ala Asn Phe Phe Pro Met Ala His Asn Thr Ala
580 585 590

Ser Thr Leu Glu Ala Met Leu Arg Asn Asp Thr Asn Asp Gln Ser Phe
595 600 605

Asn Asp Tyr Leu Cys Ala Ala Asn Met Leu Tyr Pro Ile Pro Ala Asn
610 615 620

Ala Thr Ser Val Pro Ile Ser Ile Pro Ser Arg Asn Trp Ala Ala Phe
625 630 635 640

Arg Gly Trp Ser Phe Thr Arg Leu Lys Thr Lys Glu Thr Pro Ser Leu
645 650 655

Gly Ser Gly Phe Asp Pro Tyr Phe Thr Tyr Ser Gly Ser Ile Pro Tyr
660 665 670

Leu Asp Gly Thr Phe Tyr Leu Asn His Thr Phe Lys Lys Val Ser Ile
675 680 685

Met Phe Asp Ser Ser Val Ser Trp Pro Gly Asn Asp Arg Leu Leu Thr
690 695 700

Pro Asn Glu Phe Glu Ile Lys Arg Thr Val Asp Gly Glu Gly Tyr Asn
705 710 715 720

Val Ala Gln Cys Asn Met Thr Lys Asp Trp Phe Leu Ile Gln Met Leu
725 730 735

Ser His Tyr Asn Ile Gly Tyr Gln Gly Phe Tyr Val Pro Glu Gly Tyr
740 745 750

Lys Asp Arg Met Tyr Ser Phe Phe Arg Asn Phe Gln Pro Met Ser Arg
755 760 765

Gln Val Val Asp Thr Thr Thr Tyr Thr Asp Tyr Lys Asn Val Thr Leu
770 775 780

Pro Phe Gln His Asn Asn Ser Gly Phe Val Gly Tyr Met Gly Pro Thr
785 790 795 800

Met Arg Glu Gly Gln Ala Tyr Pro Ala Asn Tyr Pro Tyr Pro Leu Ile
805 810 815

Gly Lys Thr Ala Val Pro Ser Leu Thr Gln Lys Lys Phe Leu Cys Asp
820 825 830

Arg Thr Met Trp Arg Ile Pro Phe Ser Ser Asn Phe Met Ser Met Gly
835 840 845

Ala Leu Thr Asp Leu Gly Gln Asn Met Leu Tyr Ala Asn Ser Ala His
850 855 860

Ala Leu Asp Met Thr Phe Glu Val Asp Pro Met Asp Glu Pro Thr Leu
865 870 875 880

Leu Tyr Val Leu Phe Glu Val Phe Asp Val Val Arg Ile His Gln Pro
885 890 895

His Arg Gly Val Ile Glu Ala Val Tyr Leu Arg Thr Pro Phe Ser Ala
900 905 910

Gly Asn Ala Thr Thr
915

<210> 15
<211> 607
<212> PRT
<213> Simian adenovirus

<400> 15

Met Lys Arg Ala Arg Leu Asp Asp Asp Phe Asn Pro Val Tyr Pro Tyr
1 5 10 15

Asp Thr Pro Asn Ala Pro Ser Val Pro Phe Ile Thr Pro Pro Phe Val

20 25 30

Ser Ser Asp Gly Leu Gln Glu Lys Pro Pro Gly Met Leu Ser Leu Asn
35 40 45

Tyr Gln Asp Pro Ile Thr Thr Gln Asn Gly Ala Leu Thr Leu Lys Leu
50 55 60

Gly Ser Gly Leu Asn Ile Asn Gln Asp Gly Glu Leu Thr Ser Asp Ala
65 70 75 80

Ser Val Leu Val Thr Pro Pro Ile Thr Lys Ala Asn Asn Thr Ile Gly
85 90 95

Leu Ala Phe Asn Ala Pro Leu Thr Leu Gln Ser Asp Thr Leu Asn Leu
100 105 110

Ala Cys Asn Ala Pro Leu Thr Val Gln Asp Asn Arg Leu Gly Ile Thr
115 120 125

Tyr Asn Ser Pro Leu Thr Leu Gln Asn Ser Glu Leu Ala Leu Ala Val
130 135 140

Thr Pro Pro Leu Asp Thr Ala Asn Asn Thr Leu Ala Leu Lys Thr Ala
145 150 155 160

Arg Pro Ile Ile Thr Asn Ser Asn Asn Glu Leu Thr Leu Ser Ala Asp
165 170 175

Ala Pro Leu Asn Thr Ser Thr Gly Thr Leu Arg Leu Gln Ser Ala Ala
180 185 190

Pro Leu Gly Leu Val Asp Gln Thr Leu Arg Val Leu Phe Ser Asn Pro
195 200 205

Leu Tyr Leu Gln Asn Asn Phe Leu Ser Leu Ala Ile Glu Arg Pro Leu
210 215 220

Ala Leu Thr Thr Thr Gly Ser Met Ala Met Gln Ile Ser Gln Pro Leu
225 230 235 240

Lys Val Glu Asp Gly Ser Leu Ser Leu Ser Ile Glu Ser Pro Leu Asn
245 250 255

Leu Lys Asn Gly Asn Leu Thr Leu Gly Thr Gln Ser Pro Leu Thr Val
260 265 270

Thr Gly Asn Asn Leu Ser Leu Thr Thr Thr Ala Pro Leu Thr Val Gln
275 280 285

Asn Asn Ala Leu Ala Leu Ser Val Leu Leu Pro Leu Arg Leu Phe Asn
290 295 300

Asn Thr Ser Leu Gly Val Ala Phe Asn Pro Pro Ile Ser Ser Ala Asn
305 310 315 320

Asn Gly Leu Ser Leu Asp Ile Gly Asn Gly Leu Thr Leu Gln Tyr Asn
325 330 335

Arg Leu Val Val Asn Ile Gly Gly Gly Leu Gln Phe Asn Asn Gly Ala
340 345 350

Ile Thr Ala Ser Ile Asn Ala Ala Leu Pro Leu Gln Tyr Ser Asn Asn
355 360 365

Gln Leu Ser Leu Asn Ile Gly Gly Gly Leu Arg Tyr Asn Gly Thr Tyr
370 375 380

Lys Asn Leu Ala Val Lys Thr Asp Ser Phe Arg Gly Leu Glu Ile Asp
385 390 395 400

Ser Asn Gln Phe Leu Val Pro Arg Leu Gly Ser Gly Leu Lys Phe Asp
405 410 415

Gln Tyr Gly Tyr Ile Ser Val Ile Pro Pro Thr Val Thr Pro Thr Thr
420 425 430

Leu Trp Thr Thr Ala Asp Pro Ser Pro Asn Ala Thr Phe Tyr Asp Ser
435 440 445

Leu Asp Ala Lys Val Trp Leu Ala Leu Val Lys Cys Asn Gly Met Val
450 455 460

Asn Gly Thr Ile Ala Ile Lys Ala Leu Lys Gly Thr Leu Leu Gln Pro
465 470 475 480

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Thr Ala Ser Phe Ile Ser Phe Val Met Tyr Phe Tyr Ser Asn Gly Thr
                485                 490                 495

Arg Arg Thr Asn Tyr Pro Thr Phe Glu Asn Glu Gly Ile Leu Ala Ser
            500                 505                 510

Ser Ala Thr Trp Gly Tyr Arg Gln Gly Asn Ser Ala Asn Thr Asn Val
        515                 520                 525

Thr Ser Ala Val Glu Phe Met Pro Ser Ser Thr Arg Tyr Pro Val Asn
    530                 535                 540

Lys Gly Thr Glu Val Gln Asn Met Glu Leu Thr Tyr Thr Phe Leu Gln
545                 550                 555                 560

Gly Asp Pro Thr Met Ala Ile Ser Phe Gln Ala Ile Tyr Asn His Ala
                565                 570                 575

Leu Glu Gly Tyr Ser Leu Lys Phe Thr Trp Arg Val Arg Asn Arg Glu
            580                 585                 590

Arg Phe Asp Ile Pro Cys Cys Ser Phe Ser Tyr Ile Thr Glu Glu
        595                 600                 605
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